THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FORTY-NINTH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1936.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1936, the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities	$ 910,572.21
	Real Estate	9,037.32
	Cash	1,616.08
Library Fund,	Securities	172,261.84
	Cash	21,584.91
		$1,115,072.36

The income collected from these Funds was as follows:

General Endowment	$41,941.24
Library Fund	8,251.32
	$50,192.56

an increase of more than $2,000 over the income from these Funds in 1935.

The income due from these Funds in arrears, some of which may never be collected, was on December 31, 1936

General Fund	$12,605.25
Library Fund	5,050.00
	$17,655.25

The total amount in arrears was about $325 less than on December 31, 1935.

The dividends from the General Biological Supply House have continued—the total received for the year amounting to $12,700.

Retirement Fund. A total of $4,060 in pensions was paid. The Fund at the end of the year consisted of securities of the book value of $18,923.27
Cash 684.04 $19,607.31

Income in arrears on December 31st was ..... $ 312.75

Plant Assets. The land (exclusive of Gansett and Devil's Lane tracts), buildings, equipment and library, represented an investment of $1,755,892.28
less reserve for depreciation 471,880.72 $1,284,011.56

Income and Expenses. Expenses including $41,782.21 depreciation exceeded income by $6,951.86.

There was expended from current funds net $26,319.79 for plant account.

During the year the Laboratory acquired by gift from Dr. Meigs Lot "X," Bay Shore property and the bathhouse on it and by purchase the Howes property, completing the frontage on Water Street, and the assets of the Bar Neck Corporation which included the leasehold and buildings of the Penzance Garage and the adjacent Spindell lot. At the end of the year the Laboratory owed $8,500 on mortgage on the Howes property and $8,500 in notes given for the acquisition of the Bar Neck assets. In addition it owed on notes and accounts payable $10,855.71. It had accounts and notes receivable of $14,367.37 and $14,773.65 in cash and bank accounts in its current assets.

Following is the balance sheet, the condensed statement of income and outgo and the surplus account all as set out by the accountants:

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1936

Assets

Endowment Assets and Equities:
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee—Schedules I-a and I-b $1,115,072.36
Securities and Cash—Minor Funds—Schedule II 8,298.06 $1,123,370.42

Plant Assets:			
Land—Schedule IV	$ 109,749.39		
Buildings—Schedule IV	1,238,562.84		
Equipment—Schedule IV	157,202.67		
Library—Schedule IV	250,377.38	$1,755,892.28	
Less Reserve for Depreciation		471,880.72	
		$1,284,011.56	
Cash in Dormitory Building Fund		223.24	
Cash in Reserve Fund		24.65	$1,284,259.45
Current Assets:			
Cash		$ 14,773.65	
Accounts and Notes-Receivable		14,367.37	
Inventories:			
Supply Department	$ 41,039.96		
Biological Bulletin	12,179.92	53,219.88	
Investments:			
Devil's Lane Property	$ 43,633.13		
Gansett Property	5,614.49		
Stock in General Biological Supply House, Inc.	12,700.00		
Securities and Cash—Retirement Fund—Schedule V	19,607.31	81,554.93	
Prepaid Insurance		3,293.50	
Items in Suspense (Net)		323.44	$ 167,532.77
			$2,575,162.64
Liabilities			
Endowment Funds:			
Endowment Funds—Schedule III	$1,114,980.01		
Reserve for Amortization of Bond Premiums	92.35	$1,115,072.36	
Minor Funds—Schedule III		8,298.06	$1,123,370.42
Plant Liabilities and Funds:			
Mortgage—Payable, Howes Property		$ 8,500.00	
Notes—Payable a/c Bar Neck Property Purchase		8,500.00	
Donations and Gifts—Schedule III		1,032,072.61	
Other Investments in Plant from Gifts and Current Funds		235,186.84	$1,284,259.45
Current Liabilities and Surplus:			
Accounts—Payable		$ 5,317.04	
Notes—Payable		5,500.00	
Woods Hole Oceanographic Institution		38.67	
		$ 10,855.71	
Current Surplus—Exhibit C		156,677.06	$ 167,532.77
			$2,575,162.64

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1936

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund ...		$ 41,941.24		$ 41,941.24
Library Fund		8,251.32		8,251.32
Instruction	$ 8,176.73	10,305.00		2,128.27
Research	4,146.96	14,215.00		10,068.04
Evening Lectures	88.95		$ 88.95	
Biological Bulletin and Membership Dues	9,133.99	9,087.00	46.99	
Supply Department—Schedule VI	38,262.35	43,144.91		4,882.56
Mess—Schedule VII	21,588.75	20,443.21	1,145.54	
Dormitories—Schedule VIII ..	31,984.12	11,965.84	20,018.28	
(Interest and Depreciation charged to above three Departments—See Schedules VI, VII, and VIII)	35,320.19			35,320.19
Dividends, General Biological Supply House, Inc.		12,700.00		12,700.00
Rents:				
Danchakoff Cottages	294.76	700.00		405.24
Newman Cottage	100.96	250.00		149.04
Janitor's House	21.76	240.00		218.24
Howes Property	323.37	40.00	283.37	
Bar Neck Property		2,508.74		2,508.74
Sale of Duplicate Library Sets		250.79		250.79
Interest on Notes-Receivable ..		165.00		165.00
Sundries		50.90		50.90
Maintenance of Plant:				
Building and Grounds	23,393.18		23,393.18	
Chemical and Special Apparatus	13,329.83		13,329.83	
Library Department Expense	7,753.23		7,753.23	
Truck Expense	910.27		910.27	
Sundry Expense	173.58		173.58	
Workmen's Compensation Insurance	509.71		509.71	
General Expenses:				
Administration Expenses	14,912.47		14,912.47	
Endowment Fund Trustee ...	980.29		980.29	
Bad Debts	663.53		663.53	
Reserve for Depreciation	41,782.21		41,782.21	
	$183,210.81	$176,258.95	$125,991.43	$119,039.57
Excess of Expenses over Income carried to Current Surplus—Exhibit C		6,951.86		6,951.86
		$183,210.81		$125,991.43

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1936

Balance, January 1, 1936			$152,246.38
Add:			
Reserve for Depreciation charged to Plant Funds			41,782.21
			$194,028.59
Deduct:			
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,			
Land		$10,146.34	
Buildings		13,329.91	
Equipment		5,421.95	
Library		14,757.42	
		$43,655.62	
Less,			
Notes and Mortgage payable on account of additions to Plant, Land and Buildings	$17,000.00		
Received for Plant Assets disposed of	287.50		
Adjustment of Accrued Charges on account of Library, December 31, 1935	48.33	17,335.83	
		$26,319.79	
Pensions and Allowances Paid	$ 4,060.00		
Expenses on account of Retirement Fund Securities	905.43		
	$4,965.43		
Less,			
Retirement Fund Income	$ 845.13		
Profit on Sale of Retirement Fund Securities	40.42		
	$ 885.55	$ 4,079.88	
Excess of Expenses over Income for Year as shown on Exhibit B		6,951.86	37,351.53
Balance, December 31, 1936—Exhibit A			$156,677.06

Respectfully submitted,

LAWRASON RIGGS, JR.,
Treasurer

V. THE REPORT OF THE LIBRARIAN

A report of the budget assigned to the Library by the Executive Committee for the year 1936 is as follows: books, $1,000 (with the understanding that any part of this not used for books be transferred to back sets); serials, $6,000; binding, $1,500; express, $300; supplies, $500; salaries, $7,150; back sets, $2,350; total, $18,800. The sum of $250.79 acquired by the Librarian by the sale of duplicates increased the total budget to $19,050.79. The expenditures of the Library under the same headings are itemized for the end of the year as follows: books, $695.02; serials, $5,471.00; binding, $1,614.85; express, $187.10; supplies, $386.65; salaries, $7,150; back sets, $3,478.86; total, $18,-983.48. An unspent balance of $67.31 may be accounted due to the sale of duplicates and the Librarian hopes that this sum in reverting to the general accounts may be used by the Laboratory toward securing a drying unit for the basement room in the dormitory where the Library's duplicate reprints are stored.

The acquisitions of the Library during 1936 follow: 34 back sets were completed and 20 partially completed; 29 of the completed sets were for the Marine Biological Laboratory and 5 for the Woods Hole Oceanographic Institution; 12 of the partially completed sets were for the former and 8 for the latter; the total number of current serial titles received was 1,339: 376 purchased by the Marine Biological Laboratory (19 new), 36 by the Woods Hole Oceanographic Institution (1 new), 630 by exchanges with the "Biological Bulletin" (9 new) and 57 by exchange for the publications of the Woods Hole Oceanographic Institution (18 new), 240 by gift, 224 and 16 respectively. The Marine Biological Laboratory purchased 86 new books and the Woods Hole Oceanographic Institution purchased 1, authors and publishers (authors, 8 and publishers, 26) presented 33 to the former and 1 to the latter; 30 were old books variously acquired and a fine copy of Swammerdam's "Book of Nature," 1758, was sent to the Marine Biological Laboratory by J. C. Waller of Liverpool, England, accompanied by a letter of good will to the Laboratory. The new reprints filed were 3,339 (675 current and 2,664 of previous date). Uncatalogued, and therefore omitted from this count, 580 further 1936 reprints are on hand, making the excellent total of 1,255 current reprints received before February of the following year. This is the highest record for any year in the history of the Library, and justifies the innovation of last summer in the reading-room display of current reprints. About 1,000 of the older reprints filed were from the large collection of Dr. Gilman A. Drew's reprints. Mrs. Drew presented this collection to the Marine Biological Laboratory

last summer. There remain from the valuable collection more than 6,000 reprints duplicate to the files in the Library stacks. These will be placed in the duplicate collection.

With the final statement of this report that the Library now totals 42,287 volumes and 94,980 reprints, the Librarian is under the obligation to state that the stacks for serials are filled with an allowance of growth space for each current set for the next four years (including 1937) and that the serial sets are now spread through the entire fifth, fourth and second floors and the side shelves of the third (book) floor and the first (reprint) floor. This arrangement and spacing of the serial sets was accomplished during the fall of 1936. Space for new "back sets" can be made at one end of the "book stack" for a few years, since the present book holdings may be crowded into two thirds of the space they now occupy. The reprint boxes fill the first floor space allotted to them and will be very crowded before the end of four years. All duplicate serials and reprints have now been housed outside the Library. During the year many duplicate serials and reprints were sold or exchanged. This is shown in the unusual sales and filled-in serial sets recorded above. It is necessary to explain also how 50,000 volumes, which will be the total in four years if growth occurs at the present rate of about 2,000 annually, and 108,000 reprints if each year adds 3,500, will completely fill space that in 1925 was estimated to be adequate for 100,000 volumes, or 20,000 on each of five floors. The reprint floor at once reduces the available space for volumes to a capacity for 80,000. Besides this the many serial sets and books of quarto size and over reduce the space and half of the bound serials recorded in our count are in reality two volumes bound together, so that the Library will at the end of the year 1940 actually be housing more nearly 75,000 volumes counted as volumes and not by the accession number, and 108,000 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-ninth session of the Marine Biological Laboratory for the year 1936.

1. *Attendance.* An inspection of the tabular summary of attendance on page 29 of this report and the corresponding summaries in earlier reports shows that during the past 14 years the attendance of investigators at the Marine Biological Laboratory has passed through two distinct phases and now seems to be entering into a third. The first phase was marked by a steady annual increase during which the number of

investigators grew from 176 in 1923 to 362 in 1931, the latter figure being the largest yet reached in the history of the Laboratory. In 1932, when the effects of the depression had become fully felt by most American colleges and universities, the second phase began with a sharp drop in the attendance to 314, at approximately which level it was maintained with no significant changes for four years. In 1936 with a suddenness equalled only by that of the decrease in 1932 a large increase brought the figure back almost to its highest previous level (359 in 1936 as compared with 362 in 1931). Indeed, since the number of students, which fluctuates within the rather narrow limits from year to year, happened to be at about its maximum in 1936, the total attendance of investigators and students together, after allowing for duplications, was in that year the largest in the history of the Laboratory (473 in 1936 as compared with 467 in 1931). Particularly worthy of mention is the very large number of institutions represented by students and investigators in 1936 (158 as compared with the next-highest number of 143 in 1935).

At the time of the writing of this report, though accurate figures are not yet available, it appears likely that the attendance in 1937 will surpass all previous records. If, as seems possible, the Laboratory is now entering into a new period of increasing attendance, serious consideration must be given to the best means of preventing undue crowding in the future. Since without additions to the present laboratory buildings the maximum number of investigators that can be accommodated at one time does not greatly exceed the figures reached in 1931 and in 1936, further increases will be possible only by lengthening the season and flattening the peak of attendance which now occurs early in August. That very considerable possibilities in these directions still exist is shown by the following tabulation of the attendance during the past ten years at approximately ten-day intervals throughout the working season.

		1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
May	30	7	15	9	6	6	8	11	12	11	14
June	10	50	64	55	50	51	54	46	54	43	41
"	20	114	140	139	153	153	127	129	137	127	142
"	30	212	240	197	208	217	172	184	196	174	190
July	10	247	281	238	253	258	225	235	249	226	242
"	20	247	282	242	250	273	245	253	256	232	260
"	30	245	272	249	253	281	248	255	248	257	272
August	10	234	250	256	254	302	257	261	264	245	282
"	20	208	226	243	245	280	236	244	250	235	266
"	30	168	183	220	204	239	190	205	211	192	223
September	10	110	112	157	122	136	129	117	93	94	121
"	20	50	43	59	44	69	58	45	38	26	47
"	30	12	14	14	8	14	13	12	9	11	20

2. *The Report of the Treasurer.* While the figures given above

indicate that as measured by its attendance the recovery of the Laboratory from the effects of the depression is now substantially complete, the same is not yet true with regard to its financial position, which though entirely sound, is still such as considerably to restrict its scientific activities. A study of the reports of the Treasurer for the past 6 years shows that the chief reason for this condition is the reduced income from the Endowment Fund. In 1931 the amount received from this source was $57,728.26; by 1934 the corresponding figure was $46,939.97. While it is gratifying to be able to record an increase in 1935 of approximately $1,000 above the amount received during the preceding year and a further increase of $2,000 in 1936, it is extremely unlikely, because of the low rates of interest at which funds liberated by the maturing of securities can be reinvested, that the pre-depression income from this source can be restored. Fortunately for the Laboratory, special dividends declared by the General Biological Supply House during the past two years have helped to some extent to make good the loss of income from other sources. It is also encouraging to be able to report a small but satisfactory increase in 1936 over 1935 both in the gross sales and in the profits of the Laboratory's own Supply Department.

Next in importance to the income of the Laboratory from its endowment funds is that from the fees paid by institutions and individuals for research space. That financial recovery has in this case tended to lag behind scientific recovery is shown by the fact that whereas the number of investigators in attendance in 1936 was only 3 less than in 1931 the income from the space they occupied was less by nearly $4,000. To this extent, therefore, the burden of the depression has been transferred from colleges and universities to the Marine Biological Laboratory at a time when its own income from other sources has been significantly reduced. It is encouraging to note, however, that during the past year there has been a substantial increase in the amount received by the Laboratory for research space, the figures for the years 1935 and 1936 being $12,470.00 and $14,215.00 respectively.

3. *The Report of the Librarian.* During the year covered by this report the growth of the Library has continued at a very satisfactory rate. Particularly noteworthy are the increases in the number of journals currently received (1,339 in 1936 as compared with 1,271 in 1935), the completion of a considerable number of back sets of journals, and further important additions to the reprint collection. With complete sets of almost all the more important periodicals in Biology and the related fields accessible at all times in the library itself, and with almost 100,000 reprints available for the use of investigators in their own

rooms, it can now be said without exaggeration that the biologists working at the Marine Biological Laboratory enjoy library facilities which are unsurpassed anywhere. The growth of the library since 1926 is more completely summarized in the following table:

	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Serials received currently	628	764	874	985	1,060	1,080	1,126	1,137	1,197	1,271	1,335
Total number of bound volumes	18,200	22,800	26,500	28,300	31,500	33,800	36,000	37,400	38,600	40,200	42,000
Reprints	38,000	43,000	51,000	59,000	64,000	70,000	76,000	81,000	86,000	92,000	95,000

4. *Courses of Instruction.* At its last meeting in 1936 the Executive Committee received and accepted with regret the resignation of Dr. Elbert C. Cole as head of the Course in Invertebrate Zoölogy, a position which he had filled with conspicuous success since 1932. As his successor the Committee appointed Dr. T. H. Bissonnette of Trinity College, who as a member of the Staff since 1926 and as Acting Head of the Course in 1936 is peculiarly well fitted to continue the work so ably directed by Dr. Cole and his predecessors.

5. *Lectures and Scientific Meetings.* During the past summer 13 evening lectures were given and 9 evening meetings were held for the presentation and discussion of shorter papers by investigators associated with the three Woods Hole scientific institutions. The number of papers so presented was 37; their titles are listed on page 31. In addition, the General Scientific Meeting on August 27 and 28, which was devoted exclusively to work accomplished at the Marine Biological Laboratory during the current season, was the most successful ever held. So large was the number of titles submitted that it was necessary to devote two full mornings to scientific papers and an additional afternoon to demonstrations. The program of this meeting on page 34 and the abstracts of the papers published in the Biological Bulletin for October 1936, give a very excellent picture of the work carried on at the Laboratory during the summer of 1936. In addition to its own scientific activities, the Laboratory also acted as host to the Genetics Society of America, which on September 3, 4 and 5 held in Woods Hole a very well-attended and successful meeting.

6. *Acquisitions of Property.* The past year has seen three important additions to the land and buildings owned by the Marine Biological Laboratory. The first of these, a very generous gift to the Laboratory by Dr. Edward B. Meigs, is the bathing beach and large bathhouse on Buzzard's Bay, together with the remainder of what is officially known as Lot X of the Bay Shore Property of Henry H. and Sarah B. Fay, having a total area of approximately 35,000 square feet. The beach, with a frontage of approximately 200 feet on the water, is the best one

available in the vicinity of the Laboratory, and has been used for many years by our students and investigators, though with some uncertainty as to whether this privilege might at some future time be withdrawn. The generosity of Dr. Meigs has effectively put an end to all such doubts.

A second very important acquisition, obtained through the foresight and generosity of Mr. Charles R. Crane, to whom the Marine Biological Laboratory is already so deeply indebted for support during its early struggles for existence and for very substantial aid in placing it in its present position of security, is that of the land and buildings occupied by the Penzance Garage on Main Street and the Spindell lot adjoining it on the southeast. This tract of over two acres extends from the property owned by the Oceanographic Institution to the entrance into the Eel Pond. Its fine frontage on Great Harbor offers very important possibilities for the future development of the Laboratory.

The third addition, obtained by purchase, is the Howes property on Main Street consisting of a house and barn and a lot 77 by 119 feet lying between the Kidder House and the "Homestead." The acquisition of this property completes the ownership by the Laboratory of the entire block bounded by Main and Center Streets and East and West Streets, respectively. Within these boundaries are the original laboratory building erected in 1888 and most of the wooden buildings added subsequently. The importance to the Laboratory of the possession of this unbroken tract is obvious.

7. *Board of Trustees.* At the annual meeting of the corporation held on Tuesday, August 11, Professor S. O. Mast of Johns Hopkins University was chosen to fill the vacancy in the class of 1940 created by the earlier election at the same meeting of Dr. M. J. Greenman as Trustee Emeritus.

There are appended as parts of the report:

1. The Staff, 1936.
2. Investigators and Students, 1936.
3. A Tabular View of Attendance, 1932–1936.
4. Subscribing and Coöperating Institutions, 1936.
5. Evening Lectures, 1936.
6. Shorter Scientific Papers, 1936.
7. General Scientific Meeting, 1936.
8. Members of the Corporation, August, 1936.

Respectfully submitted,

M. H. JACOBS,
Director.

1. THE STAFF, 1936

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology Emeritus, The University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy Emeritus, Harvard University.
E. B. WILSON, Professor of Zoölogy Emeritus, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Professor of Biology, Williams College. (Absent in 1936.)
C. E. HADLEY, Associate Professor of Biology, New Jersey State Teachers College at Montclair.
F. R. KILLE, Instructor in Zoölogy, Swarthmore College.
S. A. MATTHEWS, Associate in Anatomy, School of Medicine, University of Pennsylvania.
O. E. NELSEN, Assistant Professor of Zoölogy, University of Pennsylvania.
L. P. SAYLES, Assistant Professor of Biology, College of the City of New York.
A. J. WATERMAN, Assistant Professor of Biology, Williams College.

JUNIOR INSTRUCTORS

P. S. CROWELL, JR., Instructor in Biology, Brooklyn College.
A. M. LUCAS, Associate Professor of Zoölogy, Iowa State College.

PROTOZOÖLOGY

I. INVESTIGATION

(See Zoölogy)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
ELIZABETH DRUMTRA, Instructor in Zoölogy, Wilson College.
G. W. KIDDER, Instructor in Zoölogy, College of the City of New York.

EMBRYOLOGY

I. Investigation

(See Zoölogy)

II. Instruction

L. G. Barth, Assistant Professor of Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University. (Absent in 1936.)
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Tennessee.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

Robert Chambers, Professor of Biology, New York University.
J. K. W. Ferguson, Assistant Professor of Physiology, University of Western Ontario.
Rudolf Höber, Visiting Professor of Physiology, University of Pennsylvania.
Laurence Irving, Professor of Experimental Biology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
C. Ladd Prosser, Assistant Professor of Physiology, Clark University.

Junior Instructors

Kenneth Fisher, Demonstrator in Biology, University of Toronto.
F. J. M. Sichel, Royal Society of Canada Fellow.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
Francis Drouet, Research Fellow, University of Missouri.
G. W. Prescott, Assistant Professor of Biology, Albion College.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.
Edith Billings, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Manager.
Oscar W. Richards, Chemical Service.
G. Failla, X-Ray Physicist.
Thomas E. Larkin, Superintendent.
William Hemenway, Carpenter.
Lester F. Boss, Research Technician.
J. D. Graham, Glassblower.
P. H. Liljestrand, Assistant.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Doris Endrejat, Mary A. Rohan, Assistants.

SUPPLY DEPARTMENT

James McInnis, Manager.
Milton B. Gray, Collector.
A. M. Hilton, Collector.
A. W. Leathers, Shipping Department.
Geoffrey Lehy, Collector.
Walter Kahler, Collector.
Ruth S. Crowell, Secretary.
Anna N. Hall, Secretary.

MUSEUM

George M. Gray, Curator Emeritus.

2. INVESTIGATORS AND STUDENTS, 1936

Independent Investigators

Abramowitz, A. A., Research Assistant, Harvard University.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, W. C., Professor of Zoölogy, The University of Chicago.
Amberson, William R., Professor of Physiology, University of Tennessee.
Anderson, Rubert S., Research Associate, Princeton University.
Appel, F. W., Associate Professor of Biology, St. John's College.
Armstrong, Philip B., Assistant Professor of Anatomy, Cornell University Medical College.
Bailey, Percy L., Jr., Instructor in Physiology, College of the City of New York.
Ball, Eric G., Associate in Physiological Chemistry, Johns Hopkins University Medical School.

BALLARD, WILLIAM W., Assistant Professor, Dartmouth College.
BARTH, L. G., Assistant Professor of Zoölogy, Columbia University.
BAUER, HANS G. E., International Research Fellow, Rockefeller Foundation.
BERNSTEIN, FELIX, Professor of Biometrics, New York University.
BISSONNETTE, T. H., Professor of Biology, Trinity College.
BODINE, J. H., Head of Zoölogy Department, University of Iowa.
BOZLER, EMIL, Fellow in Medical Physics, Johnson Foundation, University of Pennsylvania.
BRAY, CHARLES W., Assistant Professor, Princeton University.
BRINLEY, F. J., Assistant Professor of Zoölogy, North Dakota State College.
BRONK, D. W., Professor of Biophysics, University of Pennsylvania.
BROOKS, M. M., Research Associate in Biology, University of California.
BROOKS, S. C., Professor of Zoölogy, University of California.
BROWN, DUGALD E. S., Assistant Professor of Physiology, New York University, College of Medicine.
BUDINGTON, R. A., Professor of Zoölogy, Oberlin College.
BURTON, ALAN C., Training Fellowship, General Education Board.
CABLE, R. M., Assistant Professor of Parasitology, Purdue University.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CAMERON, JOHN A., Instructor in Zoölogy, University of Missouri.
CANNAN, ROBERT K., Professor of Chemistry, New York University, College of Medicine.
CARLSON, J. G., Instructor in Zoölogy, University of Alabama.
CAROTHERS, E. ELEANOR, Research Associate, University of Iowa.
CATTELL, MCKEEN, Associate Professor of Pharmacology, Cornell University Medical College.
CHAMBERS, ROBERT, Research Professor of Biology, New York University.
CHENEY, RALPH H., Professor of Biology, Long Island University.
CHILD, GEORGE P., Research Assistant in Biology, Amherst College.
CLARK, ELEANOR L., University of Pennsylvania Medical School.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania.
CLEMENT, ANTHONY C., Assistant Professor of Biology, College of Charleston.
CLOWES, G. H. A., Director of Research, Eli Lilly & Co.
COE, W. R., Professor of Biology, Yale University.
COKER, R. E., Professor of Zoölogy, University of North Carolina.
COONFIELD, B. R., Assistant Professor, Brooklyn College.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COREY, H. IRENE, Research Assistant, University of Pennsylvania.
CO TUI, FRANK W., Associate Professor, New York University, College of Medicine.
COWDRY, E. V., Professor of Cytology, Washington University.
CROWELL, PRINCE SEARS, JR., Instructor, Brooklyn College.
CURTIS, W. C., Professor of Zoölogy, University of Missouri.
CURWEN, ALICE O., Assistant Professor of Anatomy, Woman's Medical College of Pennsylvania.
DAN, KATSUMA, Research Associate, Misaki Marine Biological Station, Misaki, Japan.
DILDINE, GLENN C., Instructor in Zoölogy, Northwestern University.
DILLER, WILLIAM F., Instructor in Zoölogy, Dartmouth College.
DONALDSON, HENRY H., Member, Wistar Institute.
DORNFELD, ERNST J., Assistant in Zoölogy, University of Wisconsin.
DREYER, WILLIAM A., Instructor in Zoölogy, University of Cincinnati.
DROUET, FRANCIS, Research Fellow, University of Missouri.
DUBUY, H., Research Fellow, Harvard Medical School.
DUNIHUE, F. W., Instructor, New York University.
ETS, HAROLD N., Associate Professor, Loyola University, School of Medicine.

FERGUSON, JAMES K. W., Assistant Professor of Physiology, University of Western Ontario, Medical School.
FIGGE, FRANK H. J., Associate Professor of Anatomy, University of Maryland Medical School.
FLEISHER, MOYER S., Professor of Bacteriology and Hygiene, St. Louis University.
FRENCH, C. S., 18 Tremont Street, Boston, Massachusetts.
FRY, HENRY J., Visiting Investigator, Cornell University Medical College.
GARREY, W. E., Professor of Physiology, Medical School, Vanderbilt University.
GERARD, RALPH W., Associate Professor of Physiology, The University of Chicago.
GILCHRIST, FRANCIS G., Assistant Professor of Zoölogy, Pomona College.
GOLDFELDER, ANNA, Research Assistant, Columbia University, Institute of Cancer Research.
GOTTSCHALL, GERTRUDE G., Assistant in Biochemistry, Cornell University Medical College.
GRAVE, B. H., Professor of Zoölogy, DePauw University.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
HADLEY, CHARLES E., Associate Professor of Biology, New Jersey State Teachers College.
HARTLINE, H. K., Fellow in Medical Physics, University of Pennsylvania.
HARVEY, ETHEL BROWNE, Princeton University.
HEADLEE, WILLIAM H., Instructor in Biology, Purdue University.
HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HENSHAW, PAUL S., Biophysicist, Memorial Hospital.
HESS, WALTER N., Head of Biology Department, Hamilton College.
HIBBARD, HOPE, Associate Professor of Zoölogy, Oberlin College.
HILL, SAMUEL E., Assistant in General Physiology, Rockefeller Institute.
HOADLEY, LEIGH, Professor of Zoölogy, Harvard University.
HÖBER, JOSEPHINE, University of Pennsylvania.
HÖBER, RUDOLF, Visiting Professor of Physiology, University of Pennsylvania.
HOLLAENDER, ALEXANDER, Special Investigator, Radiation Committee, National Research Council, University of Wisconsin.
HOLTFRETER, JOHANNES, Assistant, Zoologisches Institut, München, Germany.
HOPKINS, DWIGHT L., Assistant Professor of Zoölogy, Duke University.
HÖRSTADIUS, SVEN, Associate Professor, University of Stockholm, Stockholm, Sweden.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HUMMEL, KATHARINE P., Research Instructor, Cornell University.
IRVING, LAURENCE, Professor of Experimental Biology, University of Toronto.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JENKINS, GEORGE B., Professor of Anatomy, Medical School, George Washington University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University Medical School.
JONES, E. RUFFIN, JR., Associate Professor, College of William and Mary.
JONES, RUTH MCC., Instructor in Zoölogy and Botany, Swarthmore College.
KEIL, ELSA M., Assistant Professor of Zoölogy, Rutgers University.
KELTCH, ANNA K., Research Chemist, Eli Lilly & Co.
KIDDER, GEORGE W., Instructor, College of the City of New York.
KILLE, FRANK R., Instructor, Swarthmore College.
KINDRED, JAMES E., Associate Professor of Histology and Embryology, University of Virginia, Medical School.
KING, JESSIE L., Professor of Physiology, Goucher College.
KLEINHOLZ, L. H., Austin Teaching Fellow, Harvard University.
KNISELY, MELVIN H., Fellow, General Education Board.
KNOWER, HENRY MCE., Research Associate in Biology, Yale University.

KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, Medical College.
KOPAC, M. J., Research Associate in Biology, New York University.
KORR, IRVIN M., Procter Fellow, Princeton University.
KRAATZ, C. P., Graduate Assistant in Zoölogy, University of Cincinnati.
KRAHL, M. E., Research Chemist, Eli Lilly & Co.
KUTTNER, ANN G., Resident Physician, Rockefeller Institute.
LANCEFIELD, DONALD E., Professor of Zoölogy, Columbia University.
LANCEFIELD, REBECCA C., Associate in Bacteriology, Rockefeller Institute.
LEHMANN, JÖRGEN, Rockefeller Fellow, Rockefeller Institute for Medical Research.
LEVINE, DARWIN S., Teacher of Biology, Theodore Roosevelt High School, New York City, New York.
LILLIE, FRANK R., Professor of Embryology, Emeritus, The University of Chicago.
LILLIE, RALPH S., Professor of General Physiology, The University of Chicago.
LINDEMAN, V. F., Assistant Professor of Zoölogy, Syracuse University.
LOOMIS, W. E., Associate Professor of Plant Physiology, Iowa State College.
LUCAS, ALFRED M., Associate Professor of Zoölogy, Iowa State College.
LUCAS, MIRIAM SCOTT, 412 Tenth Street, Ames, Iowa.
LUCKÉ, BALDUIN, Professor of Pathology, University of Pennsylvania, School of Medicine.
LUYET, BASILE J., Associate Professor of Biology, St. Louis University.
LYNN, W. GARDNER, Instructor, Johns Hopkins University.
MACCARDLE, ROSS C., Assistant Professor of Biology, Temple University.
MACDOUGALL, MARY STUART, Professor of Zoölogy, Agnes Scott College.
MACLEAN, BERNICE L., Instructor in Zoölogy, Hunter College.
MAGERS, ELIZABETH, Assistant Professor of Physiology, Vassar College.
MAGRUDER, SAMUEL R., Research Associate, University of Cincinnati.
MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square College, New York University.
MARTIN, EARL A., Professor of Biology, Brooklyn College.
MARTIN, WALTER E., Instructor, Purdue University.
MAST, S. O., Department of Zoölogy, Johns Hopkins University.
MATHEWS, ALBERT P., Professor of Biochemistry, University of Cincinnati.
MATTHEWS, SAMUEL A., Associate in Anatomy, University of Pennsylvania.
MAVOR, JAMES W., Professor of Biology, Union College.
MCCLUNG, C. E., Director, Department of Zoölogy, University of Pennsylvania.
MCGREGOR, JAMES H., Professor of Zoölogy, Columbia University.
MICHAELIS, LEONOR, Member, Rockefeller Institute for Medical Research.
MILLER, FORREST W., Research Entomologist, Amherst College.
MOMENT, GAIRDNER B., Instructor, Goucher College.
MORGAN, LILIAN V., Pasadena, California.
MORGAN, T. H., Professor of Experimental Zoölogy, California Institute of Technology.
MORRILL, DR. C. V., Cornell University Medical College.
MULLER, H. J., Geneticist, Institute of Genetics, Moscow, Russia.
NABRIT, S. M., Professor of Biology, Atlanta University.
NAHM, LAURA J., Instructor in Biology, Flat River Junior College.
NAVEZ, ALBERT E., Milton Academy.
NELSEN, OLIN E., Assistant Professor of Zoölogy, University of Pennsylvania.
NONIDEZ, JOSÉ F., Assistant Professor of Anatomy, Cornell University Medical College.
NORTHROP, JOHN H., Member, Rockefeller Institute.
NUNNEMACHER, RUDOLPH F., Assistant, Harvard University.
ORR, PAUL R., Instructor in Biology, Brooklyn College.

OSTERHOUT, W. J. V., Member, Rockefeller Institute for Medical Research.
PACKARD, CHARLES, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
PAINTER, ELIZABETH E., Instructor in Physiology, University of Maryland, School of Medicine.
PARKER, G. H., Professor of Zoölogy, Emeritus, Harvard University.
PARPART, ARTHUR K., Assistant Professor, Princeton University.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POND, SAMUEL E., Technical Manager, Marine Biological Laboratory.
PRESCOTT, G. W., Assistant Professor of Biology, Albion College.
PROSSER, C. LADD, Assistant Professor of Physiology, Clark University.
PUCKETT, WILLIAM O., Instructor in Biology, Princeton University.
RAFFEL, DANIEL, Research Geneticist, Institute of Genetics, Moscow, Russia.
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RICHARDS, OSCAR W., Instructor in Biology, Yale University.
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ROOT, WALTER S., Associate Professor of Physiology, Medical School, University of Maryland.
RUGH, ROBERTS, Instructor in Zoölogy, Hunter College.
SABIN, ALBERT B., Assistant on the Scientific Staff, The Rockefeller Institute for Medical Research.
SANDOW, ALEXANDER, Instructor, Washington Square College.
SASLOW, GEORGE, Assistant Professor of Biology, New York University.
SAYLES, LEONARD P., Assistant Professor of Biology, College of the City of New York.
SCHECHTER, VICTOR, Instructor, College of the City of New York.
SCHENTHAL, JOSEPH E., Julius Friedenwald Research Fellow, University of Maryland, Medical School.
SCHMIDT, L. H., Research Fellow, Christ Hospital.
SCHMITT, FRANCIS O., Associate Professor of Zoölogy, Washington University.
SCHOTTÉ, OSCAR E., Assistant Professor of Biology, Amherst College.
SCHRADER, FRANZ, Professor of Zoölogy, Columbia University.
SCHRADER, SALLY HUGHES, Member, Natural Science Faculty, Sarah Lawrence College.
SCHWAB, JOSEPH J., Assistant in Zoölogy, The University of Chicago.
SCOTT, ALLAN C., Instructor in Biology, Union College.
SCOTT, J. PAUL, Associate Professor of Zoölogy, Wabash College.
SHAPIRO, HERBERT, Research Assistant, Princeton University.
SICHEL, FERDINAND J. M., Fellowship, Royal Society of Canada.
SICKLES, GRETCHEN R., Senior Laboratory Technician, New York State Department of Health.
SMELSER, GEORGE K., Instructor in Anatomy, Columbia University.
SMITH, DIETRICH C., Instructor in Physiology, University of Tennessee.
SMITH, H. P., Professor of Pathology, University of Iowa.
SPEICHER, B. R., National Research Fellow, Columbia University.
SPEICHER, KATHRYN G., New York City, New York.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STEIN, KATHRYN F., Assistant Professor, Mount Holyoke College.
STEINBACH, H. BURR, Instructor in Zoölogy, University of Minnesota.
STERN, KURT G., Visiting Lecturer in Physiological Chemistry, Yale University.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.

STRONG, OLIVER S., Professor of Neurology and Neuro-histology, Columbia University.
STUNKARD, H. W., Professor of Biology, New York University.
SUMMERS, FRANCIS M., Instructor in Biology, Bard College, Columbia University.
SZEPSENWOL, JOSEL, University of Geneva.
TAFT, CHARLES H., JR., Associate Professor of Pharmacology, Medical Branch, University of Texas.
TASHIRO, SHIRO, Professor of Biochemistry, University of Cincinnati, Medical College.
TAYLOR, WILLIAM RANDOLPH, Professor of Botany, University of Michigan.
TEWINKEL, LOIS E., Assistant Professor of Zoölogy, Smith College.
TRACY, HENRY C., Anatomy Department, University of Kansas.
TRAGER, WILLIAM, Assistant, Rockefeller Institute.
UHLENHUTH, EDUARD, Professor of Anatomy, University of Maryland, Medical College.
VARRELMAN, FERDINAND, American University.
WALKER, ROLAND, Instructor in Biology, Rensselaer Polytechnic Institute.
WARNER, E. O., Assistant Professor of Pathology, University of Iowa.
WARREN, MARSHALL R., Graduate Assistant in Zoölogy, University of Cincinnati.
WATERMAN, A. J., Assistant Professor of Biology, Williams College.
WEISSENBERG, RICHARD, University of Berlin.
WELLS, G. P., Lecturer, University College, London, England.
WHEDON, ARTHUR D., Professor of Zoölogy and Head of Department, North Dakota State College.
WHITAKER, D. M., Professor of Biology, Stanford University.
WILLIER, B. H., Professor and Head of Department of Zoölogy, University of Rochester.
WILSON, EDMUND B., Professor Emeritus in Residence, Columbia University.
WINTROBE, MAXWELL M., Associate in Medicine, Johns Hopkins University.
WOLF, E. ALFRED, Associate Professor of Biology, University of Pittsburgh.
WOLF, ERNST, Research Associate, Harvard University.
WOLF, OPAL M., Lecturer, Barnard College.
WOODRUFF, LORANDE L., Professor of Protozoölogy, Yale University.
YNTEMA, C. L., Instructor, Cornell University Medical College.
YOUNG, JOHN Z., Fellow, Magdalen College, Oxford, England.
YOUNG, ROGER A., Assistant Professor of Zoölogy, Howard University.
YOUNGSTROM, KARL A., Instructor in Anatomy, Kansas University.

Beginning Investigators

ALBAUM, HARRY G., Graduate Student, Columbia University.
ALGIRE, GLENN H., Graduate Student, University of Maryland, School of Medicine.
ANDERSCH, MARIE, Assistant Professor of Biochemistry, Woman's Medical College, Philadelphia, Pennsylvania.
ANGERER, C. A., Instructor, University of Pennsylvania.
ARTHUR, J. B. M., JR., Laboratory Assistant, Amherst College.
BISHOP, DAVID W., Instructor, University of Pennsylvania.
BOCSKEY, STEPHEN C., Associate Professor of Biology, University of Notre Dame.
BROOKS, JEANNE R., Oberlin College.
CHAMBERS, ALFRED H., JR., Swarthmore College.
CHURNEY, LEON, Instructor in Zoölogy, University of Pennsylvania.
COMPTON, ALFRED D., JR., Graduate Student, Yale University.
COPELAND, D. EUGENE, Biology Assistant, Amherst College.
CORSON, SAMUEL A., Research Associate, Washington Square College, New York University.
DAN, JEAN CLARK, University of Pennsylvania.

DENNY, MARTHA, Assistant in Zoölogy, Barnard College.
DONNELLON, JAMES A., University of Pennsylvania.
DORDICK, ISADORE, Graduate Student, University of Pennsylvania.
DRUMTRA, ELIZABETH, Instructor, Wilson College.
DUGAL, LOUIS-PAUL, Instructor in Biology, University of Montreal.
EMERSON, HENRY S., Graduate Assistant, Amherst College.
FISHER, KENNETH C., Demonstrator in Biology, University of Toronto.
FORMAN, RICHARD C., Graduate Student, Amherst College.
FRIEDMAN, SAM, Graduate Student, Washington Square College, New York University.
FRONCZAK, MICHAEL I., Assistant Advisor to the Department of Biology, Seton Hall College.
GREENFIELD, SYDNEY S., Graduate Student, Brooklyn College:
HALBREICH, MORTON A., Graduate Student, University of Pennsylvania.
HARRIS, DANIEL L., University of Pennsylvania.
HAWLEY, KATHARINE J., Smith College.
HENSON, MARGARET, Smith College.
HERSHKOWITZ, SOL. G., Student, New York University, College of Medicine.
HIRSCHFELD, NATHAN B., Student, Columbia University.
HOLLINGSWORTH, JOSEPHINE, University of Pennsylvania.
HORNOR, HELEN B., Assistant in Zoölogy, Barnard College, Columbia University.
HORTON, R. G., Graduate Student, Cornell University.
HUGHES, ROSCOE D., Assistant in Zoölogy, Columbia University.
HUNNINEN, ARNE V., Assistant, Johns Hopkins University.
HUNTER, FRANCIS R., Part-time Teaching Assistant, Princeton University.
HUNTER, LAURA N., Graduate Student, University of Pennsylvania.
HUTCHINGS, LOIS M., Teacher of Biology, Weequahic High School.
ITOH, HIDEGORO, Graduate Student, Zoölogical Laboratory, University of Pennsylvania.
JAILER, JOSEPH W., Teaching Fellow, Washington Square College, New York University.
JAKOBSEN, EDITH M., Science Teacher, Hawthorne High School, Hawthorne, New Jersey.
KALISS, NATHAN, Assistant in Zoölogy, Columbia University.
KAUFMAN, ALAN L., Student, Franklin and Marshall College.
KEHOE, CATHARINE E., Research Assistant, Oberlin College.
LILLY, DANIEL M., Instructor in Biology, Providence College.
LIPMAN, HARRY J., Graduate Assistant, University of Pittsburgh.
LONG, MARGARET E., Graduate Student, University of Pennsylvania.
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MARQUETTE, WILLIAM G., Graduate Student, Columbia University.
MAYO, VIRGINIA, Teacher, Chairman, Department of Science, Dana Hall School.
MAZIA, DANIEL, Harrison Fellow in Zoölogy, University of Pennsylvania.
MCBRIDE, T. F., Instructor Clinical Dentistry, School of Dentistry, University of Pittsburgh.
MENDOZA, GUILLERMO, Teaching Assistant, Northwestern University.
MOSER, FLOYD, Graduate Student, University of Pennsylvania.
NEWMAN, MORRIS, Graduate Student, University of Pennsylvania.
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ROSE, S. MERYL, Assistant, Columbia University.

ROSENFIELD, RICHARD E., Graduate Student, University of Pittsburgh.
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SEITCHEK, JOSEPH N., Graduate Student, University of Pennsylvania.
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SPECHT, HEINZ, Assistant in Physiology, New York University College of Medicine.
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WHEELER, NORMAN C., Assistant in Physiology, Purdue University.
WICHTERMAN, RALPH, Graduate Student, University of Pennsylvania.

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ARMSTRONG, LOUISE S., Research Assistant, Cornell University Medical College.
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CLARK, JOHN K., Assistant, Trinity College.
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ERLANGER, MARGARET, Agnes Irwin School.
EVANS, GERTRUDE, Research Assistant, The University of Chicago.
FENNELL, R. A., Research Assistant, Johns Hopkins University.
FIGGE, ROSALIE YERKES, University of Maryland, Medical School.
FLYNN, CARL M., Instructor, University of Maine.
FOLCH-PI, JORDI, Research Fellow, Rockefeller Institute.
FRANKENSTEIN, NORMAN L., Marquette University.
GLASSMAN, HAROLD N., Graduate Student, University of Pennsylvania.
GODRICH, JACK, Research Assistant, Columbia University.
GOFFIN, CATHERINE E., Research Assistant, Eli Lilly & Co.
GOLDIN, ABRAHAM, Laboratory Assistant, Brooklyn College.
GREEY, CONSTANCE M., University of Toronto.
GREEY, ELIZABETH L., Research Assistant, University of Toronto.
HILL, EDGAR S., Research Assistant, Washington University.
HOBSON, LAWRENCE B., Graduate Assistant in Zoölogy, University of Cincinnati.
KAYLOR, CORNELIUS T., Fellow in Biology, Princeton University.
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LEVIN, LOUIS, Laboratory Assistant, DePauw University.
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MAST, ELISABETH T., Research Assistant in Psychology, Johns Hopkins University.
MILLS, KATHARINE O., Technician and Assistant, University of Missouri.
MONKE, J. VICTOR, Teaching Fellow, University of Tennessee, Medical School.
MOORE, JOHN A., Assistant in Zoölogy, Columbia University.
MORAQUEZ, V., Barcelona Medical School, Spain.
MORRIS, MARION C., Washington University.
NESTLER, HERBERT A., Reader, Brooklyn College.

NICOLL, PAUL A., Washington University.
O'BRIEN, HELEN, University of Pennsylvania.
PAPPENHEIMER, JOHN R., Research Assistant, Harvard University.
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RICCA, RENATO A., Research Assistant, University of Pennsylvania.
ROBERTSON, KAY, Research Fellow, University of Toronto.
ROBERTSON, LOLA E., Research Assistant, New York University.
ROBINSON, R. A., University of Pennsylvania.
ROSE, EARL T., Milford, Iowa.
RUBIN, SOLOMON H., Teaching Fellow, New York University College of Medicine.
SALK, JONAS, Fellow in Chemistry, New York University.
SHAW, ISIDOR, Research Assistant, Long Island University.
SMITH, JAY A., Assistant in Department of Zoölogy, DePauw University.
STANBURY, JOHN, Student, Harvard Medical School.
STEIMAN, S. E., Boston University.
TAYLOR, JOHN F., Johns Hopkins Medical School.
THOMPSON, JAMES U., Weaver Fellow in Anatomy, University of Maryland, Medical School.
THORNTON, C. S., Assistant, Princeton University.
WEISBERG, HARRY F., Fellow in Vertebrate Zoölogy, College of the City of New York.
WIGHTMAN, JOHN C., Assistant, Brown University.
YOUNG, S. B., Technician, Rockefeller Institute for Medical Research.

Students

BOTANY

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SCHEER, BEATRICE A., Assistant in Botany, Connecticut College.
SHURTLEFF, ROSAMOND L., Student, Wheaton College.
THURLOW, MARTHA, Goucher College.
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EMBRYOLOGY

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ARTHUR, JAMES B. MCK., JR., Student, Amherst College.
BAUER, DONALD, Student, Dartmouth College.
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BROOKS, JEANNE R., Oberlin College.
CLARK, BEATRICE, Wellesley College.
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DANNER, EDWIN C., Assistant Instructor, University of Illinois.
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FROELICH, HELEN L., Science Librarian, Stephens College.

FRONCZAK, MICHAEL I., Department Adviser, Seton Hall College.
GAYER, H. KENNETH, Oberlin College.
HENSON, MARGARET, Student, Smith College.
HUMMEL, KATHARINE P., Instructor in Zoölogy, Mount Holyoke College.
JOHNSON, DAVID F., Graduate Student, New York University.
JOSLIN, STUART L., Wesleyan University.
KENNEDY, KATHLEEN M., Demonstrator, Memorial University College.
KLAMER, CHARLES H., Student, Wabash College.
KRIETE, BERTRAND C., DePauw University.
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MORGAN, THEODORE J., Fellow in Biology, Washington and Jefferson College.
PACK, VIRGINIA L., Student, Sarah Lawrence College.
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PHYSIOLOGY

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BALLENTINE, ROBERT G., Princeton University.
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CHAMBERS, ALFRED H., JR., Swarthmore College.
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GOLDWASSER, SEYMORE, Amherst College.
GROSSMAN, JACOB, Graduate Student, Columbia University.
LEWIS, LENA A., Technician, Lancaster General Hospital.
LILLY, DANIEL MCQ., Instructor in Biology, Providence College.
MAGALHAES, HULDA, Graduate Assistant, Mount Holyoke College.
MAGERS, ELIZABETH, Assistant Professor of Physiology, Vassar College.
MARGOLIS, FRED, Student, University of Pittsburgh.
MCDONALD, MARGARET R., Graduate Student, Rutgers University.
MILLS, KATHARINE O., Technician and Assistant, University of Missouri.
MORAQUEZ, VICENS, Facultad de Medicina, Barcelona, Spain.
RATNOFF, OSCAR D., Student, College of Physicians and Surgeons.
REED, EMERSON A., Graduate Student and Teaching Assistant, University of California.
RICHARDSON, ESTELLE D., Graduate Student, New York University.
ROSENFIELD, RICHARD E., Student, University of Pittsburgh.
SMITH, JUDITH D., Laboratory Assistant in Physiology, Wellesley College.
SMITH, PAUL E., Graduate Assistant, University of Rochester.
WOOD, ALBERTA BROWN, 75 East 55th Street, New York City, New York.

PROTOZOOLOGY

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FERENBACH, CARL, Student, Princeton University.
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GROSSMAN, CECELIA M., Teacher of Biology, Abraham Lincoln High School.
HUDSON, GRACE P., Hunter College.
LEE, GEORGE O., Columbia University.
LEWIS, WILMA M., Student Assistant, State Teachers College, Montclair.
MEGLITSCH, PAUL A., Student, University of Illinois.
METCALF, ISAAC S. H., Assistant in Zoölogy, Columbia University.
MITCHELL, ALISON M., Student, New Jersey College for Women.
MOCK, SARAH H., Graduate Student, Missouri University.

INVERTEBRATE ZOÖLOGY

ALLEN, THOMAS H., University of Iowa.
BABCOCK, RUTH H., Teacher, Caldwell High School, New Jersey.
BADER, JOAN E., Student, Montclair State Teachers College.
BISHOP, DAVID W., Instructor, University of Pennsylvania.
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BOWEN, WILLIAM J., Johns Hopkins University.
BURLINGTON, MARY, McGill University.
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COPELAND, DONALD E., Graduate Assistant, Amherst College.
CREGAN, SISTER MARY BERTHA, Professor of Biology, St. Xavier College.
CROASDALE, HANNAH T., Research Assistant, Dartmouth College.
DAWSON, RALPH W., Assistant Professor, University of Minnesota.
DOYLE, WINFIELD G., Oberlin College.
FABEN, ANN REED, Goucher College.
FARRADAY, CLAYTON L., JR., Swarthmore College.
GRANGER, BARBARA S., Research Assistant and Graduate Student, Mount Holyoke College.
GRAVE, CASWELL, II, Assistant, Washington University.
GROBSTEIN, CLIFFORD, College of the City of New York.
HARRIS, W. ALFRED, DePauw University.
HILL, DAVID L., Graduate Student, State University of Iowa.
HOGAN, SISTER STELLA MARIA, Professor of Biology, St. Xavier College.
HOYT, J. SOUTHGATE Y., Washington and Lee University.
HUNTINGTON, MARGARET O., Swarthmore College.
JOHNSON, REUBEN B., JR., Connecticut State College.
KIMBALL, RICHARD F., Student, Johns Hopkins University.
KNOTH, SIBYL C., Head of Science Department, Gulf Park College.
KOSTER, RUDOLF, Graduate Student, Harvard University.
LEWIS, WILMA M., Student Assistant, New Jersey State Teachers College.
MORGAN, GWENDOLYNN W., Sarah Lawrence College.
MOYER, ELIZABETH K., Graduate Assistant, Mount Holyoke College.
NORRIS, CHARLES H., Hamilton College.
POTTS, HUGH E., New York University.
RAY, DAVID T., Teacher, Johnson C. Smith University.

REED, MARY V., Instructor in Science and Mathematics, The Knox School.
ROXBY, JOHN B., JR., Graduate Student, Wesleyan University.
SARIN, LEON, Student, Colby College.
SASLOW, GEORGE, Assistant Professor of Biology, New York University.
SEATON, MARY J., Pennsylvania College for Women.
SENSENIG, WAYNE, JR., Student, Haverford College.
SHELTON, MEREDITH, Sarah Lawrence College.
SPRATT, NELSON T., JR., Instructor in Biology, Emory University.
STEVENSON, JAMES H., Assistant in Zoölogy, Oberlin College.
STOKES, MIRIAM, Graduate Assistant, Mount Holyoke College.
STUMP, ALEXANDER B., Student, University of Virginia.
SWIFT, KATHARINE W., Student, Smith College.
TWICHELL, ALLEN R., Student, Wabash College.
WATERMAN, TALBOT H., Harvard University.
WEIERBACH, LILY A., University of Pennsylvania.
WEINBERG, STANLEY L., Graduate Student, Columbia University.
WEIR, ELLEN H., Student, Wilson College.
WHEELER, NORMAN C., Assistant in Physiology, Purdue University.
WOOD, ELIZABETH C., Montclair State Teachers College.

3. TABULAR VIEW OF ATTENDANCE

	1932	1933	1934	1935	1936
INVESTIGATORS—Total	314	319	323	315	359
Independent	212	210	222	208	226
Under Instruction	73	66	49	56	76
Research Assistants	29	43	52	51	57
STUDENTS—Total	132	118	131	130	138
Zoölogy	55	54	54	55	55
Protozoölogy	16	11	11	16	17
Embryology	29	28	30	33	34
Physiology	18	19	23	20	22
Botany	14	6	13	6	10
TOTAL ATTENDANCE	446	437	454	445	497
Less Persons registered as both students and investigators	14	12	15	16	24
	432	425	439	429	473
INSTITUTIONS REPRESENTED—Total	141	120	131	143	158
By Investigators	94	92	98	111	120
By Students	76	58	75	70	77
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	–	1	1	–	2
By Students	1	2	5	3	3
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	8	5	4	7	9
By Students	1	–	1	1	5

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1936

American University
Amherst College
Atlanta University
Bowdoin College
Bryn Mawr College
College of Charleston
Columbia University
Cornell University
Cornell University Medical College
DePauw University
Duke University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Johnson Foundation
Eli Lilly & Co.
Long Island University
Memorial Hospital, New York City
Morehouse College
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Purdue University
Radcliffe College
Rensselaer Polytechnic Institute
Rockefeller Foundation
Rockefeller Institute for Medical Research
St. Xavier College
Seton Hall College
Smith College
Swarthmore College
Syracuse University
Temple University
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Iowa
University of Kansas
University of Maryland Medical School
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Wellesley College
Wesleyan University
Wheaton College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1936

Friday, June 26
Dr. A. C. Redfield ..... "The Ecological Significance of the Circulation of the Gulf of Maine."

Friday, July 3
Dr. H. K. Hartline ..... "Electrical Studies of Visual Mechanisms."

Friday, July 10
Dr. W. C. Allee ..... "Recent Studies in Mass Physiology."

Friday, July 17
Dr. G. H. Parker ..... "Neurohumors as the Means of Animal Color Changes."

Friday, July 24
Dr. C. E. Renn ..... "Problems of the Eel Grass Situation."

Friday, July 31
Dr. Sven Hörstadius ..... "Researches on Determination in the Early Development of the Sea-urchin."

Friday, August 7
Dr. J. K. W. Ferguson ..... "Newer Views of CO_2 Transport and Their Significance to Other Physiological Processes."

Friday, August 14
Dr. H. W. Stunkard ..... "Life Cycles of Digenetic Trematodes."

Wednesday, August 19
Dr. J. R. Katz ..... "Submicroscopical Structure of Living Organs (Muscle, etc.) Revealed by X-Rays."

Friday, August 21
Mr. J. Z. Young ..... "Giant Nerve Fibres in the Squid."

Monday, August 24
Dr. R. Weissenberg ..... "The Lymphocystic Disease of Fishes and Its Significance for Intracellular Parasitism: a Contribution to the Knowledge of the Virus Diseases."

Thursday, September 3 (Under the joint auspices of the Genetics Society of America and the Marine Biological Laboratory)
Dr. Th. Dobzhansky ..... "Genetic Nature of Species Differences."
Dr. Lee R. Dice ..... "Some Types of Waltzing and Epilepsy in Mice of the Genus Peromyscus." (Motion Picture.)

Friday, September 4
Dr. Johan Hjort ..... "Distribution of Marine Animals in Relation to Their Environment."

6. SHORTER SCIENTIFIC PAPERS, 1936

Tuesday, June 30
Dr. Matilda M. Brooks ..... "The Effect of Methylene Blue on the Spectrophotometric Picture of Hemoglobin, CO-Hemoglobin and CN-Hemoglobin."
Mr. Harry J. Lipman ..... "The Phosphatase Content of the Developing Chick Embryo."

Dr. Kenneth C. Fisher and
Dr. Laurence Irving ..... "The Description of an Oxidative Process Maintaining the Frequency of the Heart Beat."

Dr. Eric G. Ball ..... "Oxidation-reduction Potentials and Potentiometric Determination of Ascorbic Acid."

Tuesday, July 7

Dr. Alfred M. Lucas ..... "Nerve Cells Without Central Processes in the Fourth Spinal Ganglion of the Frog."

Dr. José F. Nonidez ..... "Receptor Areas in the Venae Cavae and the Pulmonary Veins and Their Relation to Bainbridge's Reflex."

Dr. C. Ladd Prosser ..... "Extinction of Reflex Responses in the Rat."

Dr. R. W. Gerard ..... "Factors Influencing the Electrical Activity of the Brain."

Tuesday, July 14

Dr. A. K. Parpart ..... "The Permeability of the Erythrocytes of the Ground-hog."

Dr. H. Burr Steinbach ..... "Effect of Salts on the Injury Potentials of Frog's Muscle."

Mr. Daniel Mazia and
Dr. Jean M. Clark ..... "Free Calcium in the Action of Stimulating Agents on Elodea Cells."

Dr. Kurt G. Stern and
Dr. Delafield Dubois ..... "A Photoelectric Method for Recording Fast Chemical Reactions: Application to the Study of Catalyst-Substrate Compounds."

Tuesday, July 21

Dr. W. W. Ballard ..... "Observations on Lens Regeneration in Amblystoma."

Dr. T. H. Bissonnette ..... "Fertile Eggs from Pheasants in January by Night-lighting."

Dr. Roberts Rugh ..... "A Quantitative Analysis of the Anterior Pituitary-Ovulation Relation in the Frog."

Tuesday, July 28

Dr. Gertrude Evans ..... "The Relation between Vitamins and the Growth and Survival of Goldfishes in Homotypically Conditioned Water."

Dr. C. P. Kraatz ..... "A Possible Endocrine Rôle of the Eosinophil Leucocytes in the Female Rat."

Dr. J. E. Kindred ..... "An Interpretation of the Secondary Lymphoid Nodules in the Albino Rat."

Dr. Laurence Irving ..... "Physiological Adjustments to Diving in the Beaver."

Tuesday, August 4

Dr. Ethel Browne Harvey ..... "Development of Arbacia Eggs without Nuclei: Parthenogenetic Merogony."

Mr. F. Moser ..... "Cortical Changes in Arbacia Eggs During Fertilization—A Moving Picture."

Dr. H. J. Fry ..... "Temperature Effects on Mitotic Changes in Arbacia Eggs."

Dr. Sven Hörstadius ..... "Sea-urchin Larvæ with Cytoplasm of One Species and Nucleus of Another."

Tuesday, August 11

Dr. Laura J. Nahm ..... "A Study of the Cells of the Adrenal Gland of the Ewe during Estrus and Pregnancy."

Dr. E. Alfred Wolf and
Miss Grace Riethmiller ..... "Studies in Calcification: III. The Shell of the Hen's Egg."

Dr. Alexander Sandow ..... "Diffraction Patterns of Striated Muscle and Sarcomere Behavior during Contraction."

Dr. Frank H. J. Figge ..... "The Effect of Some Oxidation-reduction Indicator Dyes (Phenol Indophenol) on the Eyes and Pigmentation of Normal and Hypophysectomized Amphibians."

Dr. Robert Chambers ..... "The Elimination of Neutral Red by the Kidney Tubules."

Tuesday, August 18

Dr. H. P. Smith and
Dr. E. D. Warner ..... "Quantitative Studies on Blood Clotting."

Dr. Alan C. Burton ..... "The Basis of the Principle of the Master Reaction in Biology."

Dr. C. S. French ..... "Efficiency of Photosynthesis in Purple Bacteria."

Dr. Alexander Hollaender ..... "Some Effects of Ultraviolet Radiation on Bacteria."

Tuesday, August 25

Dr. E. Eleanor Carothers ..... "Cellular Behavior in Abnormal Growths Produced by Irradiation of Grasshopper Embryos."

Dr. Katsuma Dan,
Mr. T. Yanagita and
Mr. M. Sugiyama ..... "The Behavior of the Cell Surface During Cleavage."

Dr. T. H. Bissonnette ..... "Modified Sexual Photoperiodicity in Ferrets, Raccoons and Quail."

DR. EARL A. MARTIN ..... "Asexual Reproduction in Dodecaceria fimbriatus."

MR. G. P. WELLS ..... "The Physiology of the Stomatogastric System in Arenicola marina."

7. GENERAL SCIENTIFIC MEETING, 1936

Thursday, August 27

MR. F. R. HUNTER AND
DR. E. N. HARVEY ..... "The Effect of Lack of Oxygen on the Permeability of the Egg of Arbacia punctulata."

DR. B. LUCKÉ,
MR. R. RICCA AND
DR. H. K. HARTLINE ..... "Comparative Permeability to Water and Certain Solutes of the Egg Cells of Three Marine Invertebrates (Arbacia, Cumingia and Chaetopterus)."

MR. S. A. CORSON ..... "Permeability of Ameba proteus to Ions."

DR. F. J. M. SICHEL AND
DR. A. C. BURTON ..... "A Kinetic Method of Studying Surface Forces in the Egg of Arbacia."

DR. R. CHAMBERS ..... "Experimental Studies on the Oil-wetting Property of the Plasma Membrane."

DR. M. J. KOPAC ..... "Interfacial Films between Oil and Cytoplasm."

DR. P. S. HENSHAW ..... "The Question of Recovery from X-ray Effects in Arbacia Sperm."

MISS ANNA K. KELTCH,
DR. G. H. A. CLOWES AND
DR. M. E. KRAHL ..... "The Respiratory Effects Exerted by Certain Organic Compounds in Relation to Their Molecular Structure."

DR. M. E. KRAHL,
DR. G. H. A. CLOWES AND
MR. J. F. TAYLOR ..... "Action of Metabolic Stimulants and Depressants on Cell Division at Varying Carbon Dioxide Tensions."

DR. W. C. ALLEE AND
DR. GERTRUDE EVANS ..... "Further Studies on the Effect of Numbers Present on the Rate of Cleavage in Arbacia."

DR. A. K. PARPART AND
DR. M. H. JACOBS ..... "Paradoxical Osmotic Volume Changes in Erythrocytes."

Dr. M. H. Jacobs,
Mr. H. N. Glassman and
Dr. A. K. Parpart ..... "Further Studies on Specific Physiological Properties of Erythrocytes."

Friday, August 28

Dr. C. C. Speidel ..... "Experiments on the Contractile Substance of Muscle Fibers." (With Motion Pictures.)

Miss Elsa M. Keil and
Dr. F. J. M. Sichel ..... "The Injection of Aqueous Solutions, Including Acetylcholine, into the Isolated Muscle Fiber."

Dr. E. Bozler ..... "The Double Refraction of Smooth Muscle."

Dr. F. O. Schmitt,
Dr. R. S. Bear and
Mr. J. Z. Young ..... "Some Physical and Chemical Properties of the Axis Cylinder of the Giant Axons of the Squid, Loligo pealii."

Mr. G. Schoepfle and
Mr. J. Z. Young ..... "The Structure of the Eye of Pecten."

Dr. H. K. Hartline ..... "The Discharge of Impulses in the Optic Nerve Fibers of the Eye of Pecten irradians."

Dr. K. C. Fisher and
Dr. J. A. Cameron ..... "The Effect of Light on the CO-poisoned Embryonic Fundulus Heart."

Dr. G. Saslow ..... "Prevention of Edema in Frog Perfusions in the Absence of Serum Proteins."

Dr. J. A. Cameron and
Miss K. O. Mills ..... "Behavior of Frog Tadpole Epidermal Cells during Seven Successive 24-Hour Regeneration Periods."

Dr. E. R. Clark and
Mrs. Eleanor L. Clark ..... "Observations on Conditions Affecting Growth of Cells and Tissues, from Microscopic Studies on the Living Animal."

Miss Laura N. Hunter ..... "Some Nuclear Phenomena in the Trichodina (Protozoa, Ciliata, Peritrichida) from Thyone briareus (Holothuroidea)."

Dr. S. Hörstadius ..... "Investigations on Determination in the Early Development of Cerebratulus."

Dr. R. Rugh ..... "Preliminary Evidence as to a Source of the Growth and the Sex-stimulating Hormones in the Bullfrog."

Dr. P. B. Armstrong ..... "Mechanism of Hatching in Fundulus heteroclitus."

Dr. B. H. Grave and
Mr. J. Smith ..... "Hermaphroditism and Sexual Inversion in Mollusca."

PAPERS READ BY TITLE

Dr. H. G. duBuy ..... "Separation of the Conducting and Contractile Elements in the Retractor Muscle of Thyone briareus."

Dr. A. V. Hunninen and
Dr. R. Wichterman ..... "Hyperparasitism: A Species of Hexamita (Protozoa, Mastigophora) Found in the Reproductive Systems of Deropristis inflata (Trematoda) of Marine Eels."

Dr. Mary S. MacDougall ..... "Cytological Studies of the Genus Chilodonella. III. The Conjugation of Chilodonella labiata, variation?"

Dr. Victor Schechter ..... "Comparative Hypotonic Cytolysis of Several Types of Invertebrate Egg Cells and the Influence of Age."

Dr. H. W. Stunkard ..... "Notes on Life Cycles of Digenetic Trematodes."

Dr. A. J. Waterman ..... "Inhibition of Gastrulation in Arbacia with $NiCl_2$."

Dr. R. Wichterman ..... "Division and Conjugation in Nyctotherus cordiformis (Ehr.) Stein (Protozoa, Ciliata) with Special Reference to the Nuclear Phenomena."

DEMONSTRATIONS

Thursday, August 27

Dr. F. H. J. Figge ..... "The Effect of Some Indophenol Dyes on the Eyes and Pigmentation of Various Amphibian Larvæ."

Dr. R. Weissenberg ..... "Intracellular Parasitism of Microsporidia and of Lymphocystic Disease."

Dr. S. E. Pond	"A Semi-portable Cold Room Providing Daylight and Automatic Adjustment of Temperature from —10° C. to Room Temperature."
Miss Laura N. Hunter	"Some Nuclear Phenomena in the Trichodina from Thyone briareus (Vegetative Stages, Binary Fission, Conjugation (?))."
Dr. A. Hollaender	"A Simple Intense Mercury Vapor Lamp."
Dr. Ethel B. Harvey	"Cleavage of Non-nucleate Arbacia Eggs."
Dr. E. R. Clark and Mrs. Eleanor L. Clark	"Observations on Conditions Affecting Growth of Cells and Tissues, from Microscopic Studies on the Living Animal."
Miss Gertrude Gottschall	"The Determination of Glutathione in Animal Tissues."
Dr. D. Marsland	"A Bomb Permitting the Microscopic Observation of Cells and Tissues during Hydrostatic Compression. The Effects of Compression on the Cleavage of Arbacia Eggs."
Dr. S. E. Hill	"Action Currents in Nitella."
Mr. F. R. Hunter	"Apparatus for Determining Volume Changes of Cells under Anærobic Conditions."
Dr. H. W. Stunkard	"Life Cycles of Digenetic Trematodes."
Mr. J. Z. Young	"Anatomy and Physiology of Giant Nerve Fibers in the Squid."
Dr. E. Bozler	"Method Recording Changes of Double Refraction during Muscular Contraction."
	"Sensitive Method of Recording Responses of Blood Vessels."
Dr. H. K. Hartline	"The Discharge of Impulses in the Optic Nerve Fibers of the Eye of Pecten irradians."
Dr. M. N. Knisely	"Microscopic Demonstration of Several Living Internal Organs of Frogs Illuminated with Fused Quartz Rods."

8. MEMBERS OF THE CORPORATION

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PLACOID SCALE TYPES AND THEIR DISTRIBUTION IN SQUALUS ACANTHIAS

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Detailed studies of the development of placoid scales were reported by Hertwig (1874). His descriptions of certain integumentary scales and of stomodeal denticles have been supplemented by Steinhard (1903), Imms (1905) and Radcliffe (1916). These workers give no indication that *Squalus* has more than a couple of different types of integumentary scales or that scaleless regions occur. Most text-book statements imply that dogfish scales all have pointed spines. For instance, Wilder (1923, p. 82) says: "The scales in the dogfish are of the form known as placoid, each consisting of an approximately flat base from which rises a sharp-pointed cusp, inclined in the direction of the free edge of the scale, or posteriorly when the scale is in place." Sakamoto (1930), however, has reported several different shapes of scales in five Japanese species of sharks.

The work reported here was undertaken after it had been noted that there was considerable variation in the scales of pieces of dogfish skin taken at random for demonstration to students. An attempt is here made to describe the types, distribution and orientation of scales of specimens of *Squalus acanthias* of 54 to 62 cm. length.

For this work, the skin was removed in relatively large pieces, the exact orientations and positions of which were noted. In all cases the same regions of both sides of the body were compared. Detailed studies of 7 specimens were made. Several other fish were used as additional checks of certain points. At first these were studied after clearing in methyl salicylate without staining. Later several were prepared by staining in alizarin red S and then clearing. In addition small portions of the skin, representative of different scale-types, were macerated in 0.5 M NaOH at a temperature of about 85° C. Isolated scales were thus obtained.

Observations

For purposes of convenience the integument may be considered as belonging to the following regions, each of which will be considered

separately: trunk and tail regions of the body proper, as contrasted with head; fins, including spine pockets and, in male, claspers; head; mouth opening and labial pouches; ampullæ of Lorenzini; olfactory sacs; eyelids; spiracles; external gill slits; internal gill slits; mouth cavity and pharynx.

The placoid scale of *Squalus acanthias* consists of a basal plate and spine (as noted by Hertwig and many others). The basal plate (except in the case of stomodeals and combs) is quadrangular with two angles extending laterally, one anteriorly and a fourth posteriorly. The stomodeal basal plate also usually bears four extensions, but the lateral ones, as well as the anterior, extend anteriorly. The basal plate of the comb type is similar to that of stomodeals but the angles are often indefinite and the whole basal plate frequently lacks the regularity usually found in this part of other scales.

The spine may be interpreted as consisting of two main elements:

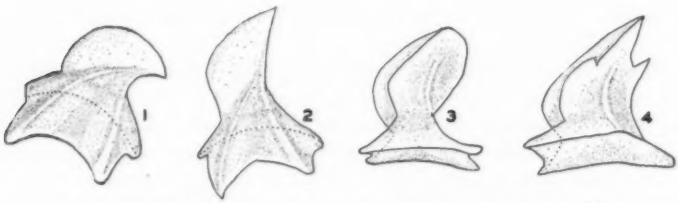


FIG. 1. Left lateral aspect of a dorsal body type scale. × 75.
FIG. 2. Right lateral aspect of a ventral body type scale. × 75.
FIG. 3. Postero-lateral aspect of a transitional type scale. × 75.
FIG. 4. Postero-lateral aspect of a body tricuspid scale. × 75.

(1) a longitudinal plate which extends, in plan, between anterior and posterior angles of the basal plate; and (2) a transverse plate which extends between the lateral angles of the basal plate. The transverse component is ordinarily tilted posteriorly and rests on the posterior part of the longitudinal component. The anterior part of the latter extends onto the transverse element at least to some extent. Scale types are associated with more or less marked variations in the development and shapes of these spine components.

It is well known that an opening on the under side of the basal plate connects with a system of dentinal canals which extend even into the spine. The openings and canal systems are not shown in the drawings.

Trunk and Tail Regions of Body Proper

On the trunk and tail regions of the body proper there are four general types of scales: (1) dorsal body type (helmet-scale of Hertwig);

(2) ventral body type; (3) transitional type; (4) tricuspid type, showing various degrees of development of the lateral cusps. These divisions are not sharp, many intermediate forms occurring.

In the dorsal body type (Fig. 1) the transverse component is more than half as wide as the basal plate; the part of the longitudinal element anterior to the transverse element is of about the same width as the latter; the posterior part of the longitudinal element is small; and the entire spine is about as high as it is wide. In the ventral body type (Figs. 2 and 14) the transverse component and the anterior part of the longitudinal component are about two-thirds as wide as in the dorsal type; the height of the spine, on the other hand, is about fifty per cent greater than in the dorsal type. The so-called transitional type (Fig. 3) is intermediate between dorsal and ventral types. The tricuspid scales (Figs. 4 and 18) are of about the same proportions as the dorsal and transitional types but bear two lateral, secondary cusps set some distance anterior to the tip of the scale and varying greatly in size in different scales.

Along the mid-dorsal line there are several (3–5) rows of large, heavy scales in which tricuspids predominate but occasional dorsal body scales are present. The dorsal and dorso-lateral surfaces bear dorsal body type and tricuspids. The latter vary from scales with large secondary cusps to some with these so small that the scales may be interpreted as intermediate between tricuspids and dorsal body type. From the level of the lateral line through the region of reduction in integumentary pigmentation, the dorsal body type grades over, through the transitional type, to the ventral body type (Fig. 16). In this transition zone the tricuspids become more and more scarce until, in the practically white ventral part of the body, nearly all scales are of the ventral body type. In this transition zone the basal plates are about twenty per cent smaller, both in length and in width, than they are either dorsally or ventrally. This smaller size, coupled with a slightly greater scattering of scales in the transition zone, gives the impression that there is a relatively great reduction in the number of scales here.

Neither the type nor the arrangement of scales is particularly altered along the lateral line (Fig. 16). Also no significant size-differences exist between anterior and posterior levels.

Body scales all point posteriorly. Frequently several may be somewhat deflected so that the long axis is slightly oblique. The general pattern of distribution shows the scales to be arranged in diagonal rows, the scales of each row lying between and behind two of the preceding row (as noted by Klaatsch, 1890, and many others). Under high magnification, however, it is at once evident that the scales are not arranged

in a precise geometric fashion. This is due to the fact that the rows are not exactly parallel, the scales vary in size, and " extra " scales are quite numerous, thus disarranging the pattern.

Fins

The *pectoral fin* will be described in detail first. The other appendages will then be described only in so far as they differ from the pectorals.

The pectoral fin has a heavy anterior margin, a thinner axillary one and a curved, filamentous distal one. The anterior and axillary extreme margins, where the skin of the dorsal side of the fin passes over into that of the ventral, both bear scales. The anterior border is covered by closely arranged and frequently overlapping fin-marginal scales (Figs. 5 and 19). Each of these scales is relatively large with a large, high

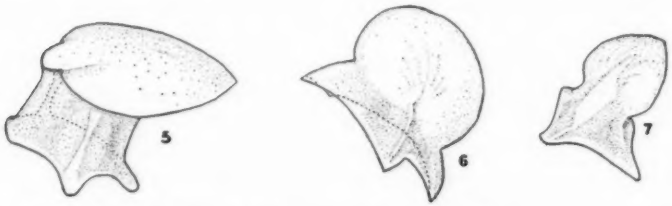


FIG. 5. Lateral aspect of a fin-marginal scale. × 75.
FIG. 6. Marginal type scale from vicinity of axillary scaleless area. × 75.
FIG. 7. Paramarginal scale from region slightly farther from axillary scaleless area than that occupied by scale in Fig. 6. × 75.

pedicel. The massive, transverse spine-component is tilted so far that its surface is nearly parallel with the surface of the integument. The posterior tip of this is only slightly pointed. A short, thick part of the anterior longitudinal component projects from the anterior end of the transverse element. Toward the distal end of the fin the spines of these scales become smaller and somewhat less massive. Extending from the entire length of this anterior border toward the middle of the fin there is a zone of transition to the common fin type which is similar to the dorsal body type but with the average scale smaller. Following through this zone from the anterior border, we find first the fin-marginals more disperse than on the border, then transitionals and body tricuspids appearing, and finally the common fin type. This transition zone extends onto the body of the fish a very short distance.

The arrangement of scales on the axillary border is complicated somewhat by the presence of a scaleless area at the base of the fin here.

The extent of this area is less on the fin than on the body wall. It is shaped somewhat like a low, thick *J* with the long part extending along the dorsal side of the fin-body junction, the loop around the posterior side and the short part on the ventral side for a short distance. This scaleless area is surrounded by several rows of marginal scales (Figs. 6 and 17). These scales have a large, rounded transverse component with the longitudinal component reduced posteriorly and almost lacking anteriorly. Next to these scales are rows of paramarginals (Fig. 7) with smaller and less rounded transverse components than have the marginals. Both of these types are more numerous on the body than on the fin. They are also less numerous at the anterior ends of the scaleless area where they may even be lacking. At the anterior ends of the ventral and dorsal parts of this area the spines point considerably away from it. In the posterior part they return gradually to their ordinary orientation. This arrangement is, however, subject to some variation. Several spines may point toward the scaleless area, for example. The basal part of the axillary border of the fin has paramarginal scales for a short distance, always greater on the dorsal side than on the ventral, probably associated with the difference in extent of the scaleless areas on the two sides. The remainder of the axillary border is covered with scales similar to those on the anterior but slightly smaller. The zone of transition toward the common fin type is narrower here than at the anterior border.

The general transition from body to fin is not associated with a change in scale type. The dorsal body type scales, found here, are smaller than on the body wall. This is especially true near the distal margin of the fin. Most of the fin is covered by this common type, more closely set than on the body wall and arranged in a more regular pattern. The scales extend to the very edge of the filamentous border which is covered by small transitional and common fin types and, at the very edge, tricuspids. These tricuspids are slightly larger than other scales here.

The scales of the anterior and axillary borders curve to follow these borders but not exactly so. The result is that the spines at the very edges extend off the margins. Except at the borders, the orientation on the fin does not follow the curvature of the fin rays but is in straight lines toward the filamentous border.

On the *anterior* and *posterior dorsal fins* the transition zone at the anterior margin extends onto the body anterior to the spine pocket which is covered with massive, shingling fin-marginals. The anterior margin of the fin is devoid of scales immediately behind the basal portion of the spine (Fig. 19). This small scaleless area is surrounded by marginal or paramarginal scales. There is also a scaleless area at the poste-

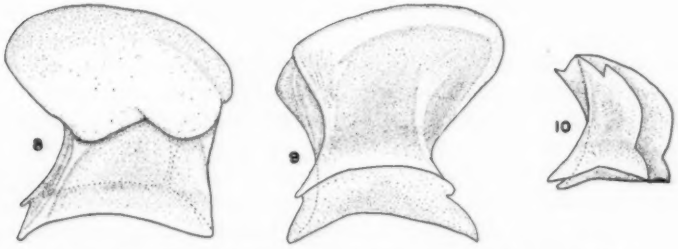
rior attachment of the fin. This area is small, symmetrical, with rounded margins, and surrounded by marginal and then paramarginal scales.

On the *pelvic fins* the fin-marginal scales are less massive than on other fins and the median borders are almost entirely covered by paramarginal scales. The cloaca is scale-free. There is a large scaleless area extending around the cloacal opening and continuing along the attachments of the median borders of the fins and onto their dorsal surfaces which are in contact with the body wall. This area does not extend onto the ventral surfaces of the fins. Around this scaleless area are marginal and then paramarginal scales. In the male the ventral side of each clasper bears marginal scales at its posterior end. The anterior end bears marginals medially, tricuspids laterally and paramarginals in transition between them on the ventral side. These scales all point medially and are closely arranged. The dorsal side of each clasper bears no scales, this scaleless area being continuous with that around the cloaca.

The *caudal fin* has no scaleless area associated with it. Transitional and tricuspid scales are more numerous than on other fins, especially toward the dorsal side.

Head

The snout is covered by a characteristic snout type scale (Figs. 8, 9 and 15). Each of these scales, like fin-marginals, is relatively large with a large, high pedicel and with a massive transverse spine-component



FIGS. 8 and 9. Different aspects of two snout type scales. × 75.
FIG. 10. Postero-lateral aspect of a head tricuspid scale. × 75.

tilted so that its surface is nearly parallel with the surface of the integument. The entire margin of this component is robust and the posterior end is rounded. In many cases there is a distinct overlapping of these scales. All snout scales radiate from a point at the extreme anterior tip of the snout but slightly dorsal. Laterally this type extends farther to

the posterior and is also more numerous than on either dorsal or ventral surfaces. These scales extend posteriorly for several millimeters where they merge into the head type (Figs. 10 and 20). Scales of the latter type are thick-set and have relatively short tricuspid spines with secondary cusps nearly as large as the main one. They become somewhat smaller and less numerous posteriorly. Near the dorsal midline they are oriented with the spine pointing directly toward the posterior. On either side of this region the spines have a slightly lateral orientation. At the level of the spiracle the head type is mixed with the dorsal body type. The spiracle opening, however, is usually surrounded by the head type for the most part. At the level of the first functional gill slit the transition to body type is usually complete. On the ventral side there are, in addition to the head type, scales similar to the snout type but less massive. The region of transition to ventral body type, on the ventral side, is relatively broad and is complicated by the presence of the mouth opening. The transition is completed a short distance posterior to the lower jaw.

Mouth Opening and Labial Pockets

There is usually a very narrow scaleless area just outside the rows of teeth. This is frequently absent near the mid-line but widens out toward the angles of the jaws. In the upper jaw region the integument adjacent to this scaleless area possesses one or two rows of marginal scales anterior to which are several rows of the paramarginals. These scales have interrupted those of the ordinary head type which are prevalent anterior to them. On the lower lip is a row, or two, of scales quite similar to the snout type, posterior to which scales of the head type occur again. Scale orientation is not affected by the presence of the lips.

The walls of the labial pouches are scaleless. No marginal or snout-type scales bound this area. Instead, the same type of scale is found here as occurs in this general region of the head, namely: paramarginals bounding the anterior part of the pouch adjacent to the upper jaw and head type along the posterior part. Apparently the only modification in scales in the vicinity of these pouches is in their orientation which is changed so that the spines follow the margins of the pouches and point postero-laterally.

Ampullæ of Lorenzini

The presence of the numerous openings of the ampullæ of Lorenzini on the head does not bring about any distinct local changes in scale-type (Fig. 20). The spines are often shaped to curve about the margins of

the openings when parts of the basal plates would ordinarily be in the area which the openings occupy. This condition is more common dorsally than ventrally. Often a spine overlaps an opening.

Olfactory Sacs

There is usually no modification of scale type about the margin of an olfactory opening. Head type scales are found here. The scales do not end abruptly but continue into the cavity a short distance. Posterior orientation is maintained mediad but not laterad to the opening. Some of the scales antero-lateral to the opening are turned medially toward the anterior flap, others laterally to follow the lateral margin of the aperture. The anterior flap is covered by scales similar to the head type but considerably smaller and more deeply serrate. They are very closely arranged and point posteriorly. The smaller posterior flap is covered by similar scales, somewhat larger, closely arranged and pointing posteriorly.

Eyelids

The dorsal and ventral eyelids do not differ as far as the scales are concerned. The scales covering the margin of an eyelid are of several types. Part of the lid is covered by head type and part by a mixture of small transitional and tricuspid scales. These scales are smaller than those of the surrounding area and much more closely arranged, being almost shingled. The scales curve about the margins, especially anteriorly. The scales are confined to the external margins, none being found on the edge of the conjunctiva.

Spiracles

The external opening of the spiracle is entirely surrounded by head type scales, smaller and more closely grouped than elsewhere in that vicinity. At the anterior side the scales are deflected away from the middle of the opening. In those cases where the head-body transition extends to this level the spiracle may be surrounded by the several types. No integumentary scales enter the opening.

The posterior wall of the spiracular cavity bears numerous typical stomodeal denticles (Fig. 11) somewhat like those described by Hertwig, Steinhard, and others. Following anteriorly around the wall, along the dorso-medial side, the denticles become less numerous up to the middorsal part, then more abundant until they are usually almost as abundant in the anterior wall as in the posterior. The ventro-lateral wall is

without denticles. There is no particular transition into this area from either anterior or posterior sides. Instead, the denticles stop rather abruptly. Also the denticles are less numerous near the external opening which they approach very closely. The points of these scales are directed toward the pharynx.

External Gill Slits

The scales covering the distal, exposed ends of the gill septa are of several types, dependent on the location of the septum. The scales covering the whole anterior surface of the first flap—in front of the first functional gill slit—are of the type on the body wall nearby but smaller. They are usually of dorsal body type, but sometimes transitionals. The posterior surface of the septum is scaleless nearly to the gill filaments. A narrow area adjacent to the filaments has comb type scales (Figs. 12 and 13) such as have already been described by Steinhard (1903). These are very few in number and haphazardly arranged.

The distal, exposed ends of the septa of the four holobranchs are alike. The anterior surface, at its free border, is covered usually by dorsal body type scales, often interspersed with transitional and tricuspid types, sometimes with small head type or paramarginals. There is a transition through paramarginals to marginals internally up to the point where the septum is covered by that in front. Then there is a scaleless region. Beyond the latter there are comb scales similar to those on the posterior face of the first flap. The posterior faces of these septa are

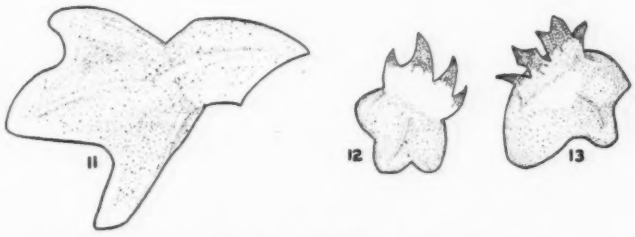


FIG. 11. Stomodeal denticle from floor of pharynx. × 75.
FIGS. 12 and 13. Two comb scales from wall of a gill slit. × 75.

similar to that of the first. There are wider areas bearing comb scales on the anterior than on the posterior faces, possibly associated with the fact that anterior demibranchs do not extend externally as far as do posterior ones. The extra part of the smooth septum in each case bears comb scales. No scales occur on the gill filaments.

The posterior wall of the fifth gill pocket bears no filaments and no comb scales near the surface of the body. A few, scattered, marginal scales are found in the outer part of the area covered by the flap of the last holobranch. Where exposed, really the general surface of the body at that point, there are at first marginal scales. The latter merge posteriorly into typical fin-marginal scales, probably associated with the adjoining pectoral fin.

Internal Gill Slits

The scales on the membrane which covers each gill arch are of two distinct types (as reported by Steinhard, 1903). One, a typical stomodeal denticle, is found toward the pharyngeal side of the arch and on

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PLATE I

Surface view photomicrographs of pieces of integument of a *Squalus acanthias* 55 cm. long. In all cases the top of the figure is the anterior end and the spines point toward the bottom of the page. Figure 17 is about × 50, all others about × 40.

FIG. 14. From the ventral, mid-trunk region. The basal plates show as gray backgrounds for the spines. Compare Fig. 2.

FIG. 15. From dorsal side of snout near point from which all scales radiate. The "heart-shaped" surface of one scale—in the lower right quarter—has been outlined. The limits of another—near the center—have also been indicated. Overlapping obscures to some extent the limits of many scales. Slightly to the left and below the center of the figure there is a scale with the anterior parts of the pedicel and basal plate clearly outlined as they recede from the notched anterior margin of the scale surface. Compare Figs. 8 and 9.

FIG. 16. From the lateral line area in the mid-trunk region. Two openings of the lateral line canal show in the center.

FIG. 17. From the side of the trunk in the axillary region. At the right side of the top of this figure the very edge of the axillary scaleless region shows. A scale in the lower right quarter has been outlined in stipple. The solid black areas are the basal plates. Compare Fig. 6.

FIG. 18. From the dorso-lateral part of the mid-trunk region. The two scales in the upper corners are slightly modified dorsal body type. All others are tricuspids. To the right of the center is one which has been outlined in stipple. Compare Fig. 4.

FIG. 19. From the region immediately distal to the scaleless area behind the base of the spine of the antero-dorsal fin. The very tip of this scaleless area shows at the center of the top edge of the figure. Two scaleless patches also show, one in the center and the other near the bottom. This strip shows the transition from the ordinary marginal type of scale—at the top—to the heavy fin-marginal type. In the lower left corner is one of the latter which shows clearly the ramifications of the portions of the pulp cavity which extend into the "heart-shaped" outer part of the spine. Compare Fig. 5.

FIG. 20. From the dorsal side of the head just anterior to the eyes. The opening of an ampulla is shown at the top. Note that the spines of scales around the opening are still oriented posteriorly. In the lower right corner a scale has been outlined. To the left of the latter the general form of a head tricuspid shows clearly. Compare Fig. 10 and also body tricuspids in Fig. 18.

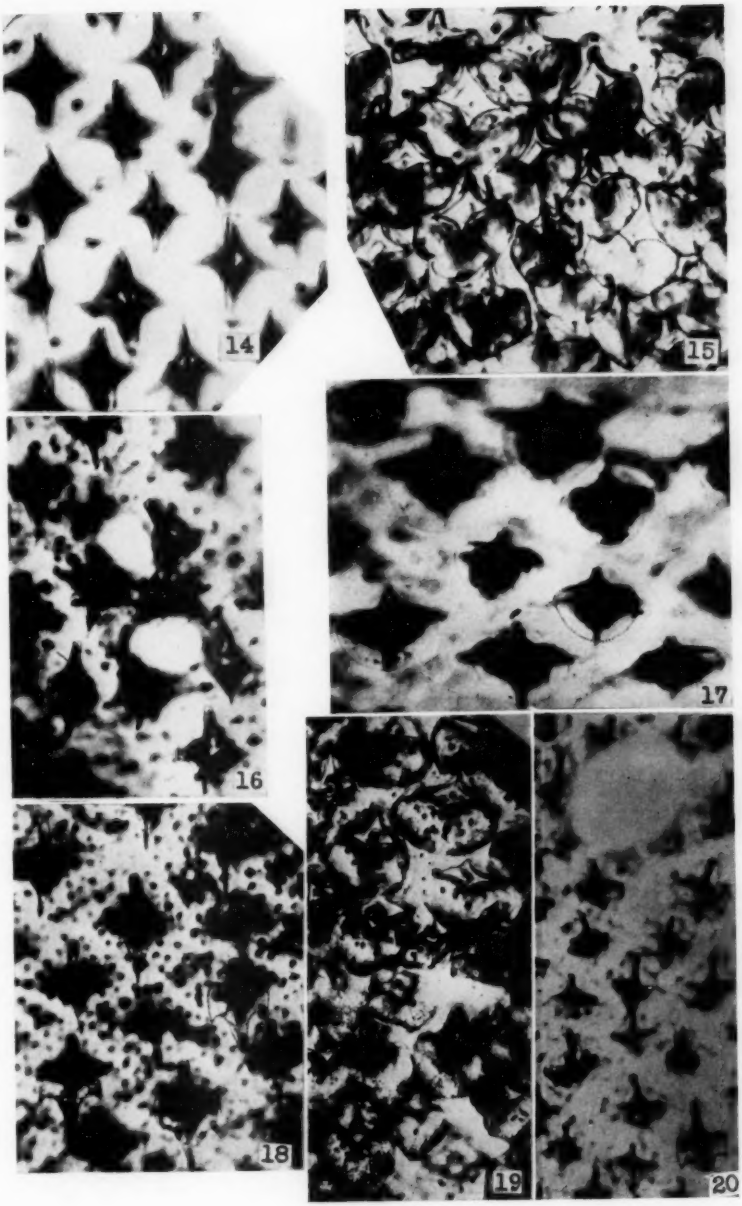

PLATE I

the gill rakers. These scales are never very numerous, but are more abundant on the anterior than on the posterior side. Usually there are more toward the dorsal than toward the ventral end of the arch and more on the rakers than on the adjacent portions of the arch. The spines point toward the pharynx. There is no distinct arrangement plan of the scales.

The second type is the comb scale, similar to that found just external to the filaments. These scales are smaller than the stomodeals and very much more numerous. They occur on the side of the arch toward the filaments, being almost exclusively confined to the anterior surface along its whole length. When present on the posterior surface, they are confined to two small areas—one dorsal and one ventral—where the membrane of the anterior side of one arch passes over into that of the posterior side of the arch in front.

This situation holds true for all four holobranchs. The anterior wall of the first functional gill slit bears only combs at the extreme dorsal and ventral ends, as mentioned for the other slits. The posterior wall of the fifth slit bears no filaments but does have many comb scales, not only in the inner part but also scattered over much of the area which corresponds to that occupied by filaments in other slits.

As with the stomodeal type, the combs all point toward the pharynx. They are usually several rows wide (4–6), frequently with each scale lying behind and between two others. This arrangement is not precise as the rows are somewhat irregular, the sizes of the scales vary and " extras " occur.

Mouth Cavity and Pharynx

There are no scales on the roof of the mouth or pharynx. The few scales on the dorso-lateral part of the pharynx are associated with the membrane covering the gill arches rather than that of the pharyngeal roof. The floor of the pharynx bears typical stomodeal denticles in a restricted area which begins in the mid-ventral line at the level of the first functional gill slit, or slightly anterior to it, and extends as far posterior as the last slit. This area is usually quite narrow near its anterior end but is about half the width of the floor of the pharynx throughout most of its length. The lateral quarter of the floor, on each side of this scale-bearing region, is ordinarily scaleless. In some specimens a few scales may be present in the posterior parts of these lateral areas. These stomodeal denticles are much sparser than body scales and are irregularly oriented, although the majority point posteriorly. They are haphazardly arranged.

Discussion

Most descriptions of the integumentary scales of *Squalus acanthias* have been confined to two types: (1) the general body type, mainly of the dorsal variety; and (2) the tricuspid, also called tridentate. Radcliffe (1916) described but one type in the integument of this animal, namely, the tridentate. In his Fig. 20 he showed twenty-one scales, all obviously of this type. This is the type which would ordinarily be present in a piece " from the middle of the side below the first dorsal fin," which Radcliffe—in a later paper (1917)—suggested using for identification purposes. These scales resemble closely those of *Scyllium* described by Klaatsch (1890) whose sketches have been used frequently as a source in depicting placoid scales. The general body type of *Squalus* was early described by Hertwig (1874) and later by Steinhard (1903). We have observed scales of both of these types in considerable numbers on the trunk and tail regions of this animal. The body type really exists in two forms, both of which have similar basal plates. The ventral body type, however, differs from the dorsal in having a considerably higher spine, both components of which are much narrower than in the dorsal type.

In addition to these two types, we have observed at least two other main types. One of these is a large scale with large pedicel and massive transverse spine-component the surface of which lies nearly parallel with the body surface. The other is a marginal type consisting of a stellate base, an abbreviated pedicel and a spine made up, for the most part, of a large, circular transverse-component, the longitudinal elements being much reduced. In addition there are scales of several other shapes which should perhaps be regarded as intermediates between other types since they are found in places which may be considered transition zones.

The massive scales are apparently of two distinct varieties. One is the so-called snout type found on the tip of the snout and on the tip of the lower jaw. The other, the fin-marginal, is on the anterior margins of all fins except the parts of the antero- and postero-dorsals which are protected by the fin-spines. Both of these varieties tend to be set close together and to overlap in many cases. It would appear that this is a special protective type found especially on anteriorly exposed surfaces.

The ordinary marginal scales surround scaleless areas where one part of the actual surface of the body covers another. Such areas include: those in the two axillary regions (on the body wall and on the base of each pectoral fin along the posterior portion of the attachment of the latter); those beneath the posterior portions of the two dorsal

fins; those behind the spines of these dorsal fins; those beneath the external flaps of the gill septa; and a very large area along the dorsal sides of the pelvic fins, on adjacent parts of the body wall and around the cloaca. No mention of these scaleless areas has been made by other workers, including Sakamoto (1930), who made a thorough study of several species of Japanese sharks. On the other hand, those scaleless areas which are inside of various invaginations, in most pores and in other openings are usually not surrounded by special marginal scales but instead by the type commonly found in the region in question. Included among these places are the ampullæ of Lorenzini (Fig. 20), the pores of the lateral line canals (Fig. 16) and associated canals of the acusticolateral system of the surface of the head, the labial pockets, the spiracles, the olfactory sacs, and the inpocketings to form the eyelids.

Sakamoto (1930) has depicted a number of types of scales some of which resemble certain of these of *Squalus*. In describing the scales of *Cynias manazo*, he reported that the " ridges of the scales on the dorsal side of the trunk are more massive and higher than those of the scales on the ventral side of the same." In *Squalus* there exists a similar condition of the single ridge which we have called the anterior part of the longitudinal spine-component. None of the scales of *Squalus* seem to bear more than a single such ridge. Rudiments of this element are present on most scales of *Squalus*. They were confined to the trunk and caudal scales of four of the five species studied by Sakamoto. In *Carcharinus japonicus*, ridges were also present on scales of the buccal cavity. This worker also reported that in general in *Cynias* " the width of the basal plate (is) proportional to that of the whole placoid scale." In *Squalus* such is not the case. For example, the spines of ventral trunk scales are much more narrow than those of the dorsal type. The basal plates, however, are of approximately the same size and shape in both types. Also, in the lateral transition zone, where the spines are intermediate in size, the basal plates are considerably smaller than those of either dorsal or ventral scales.

Sakamoto also found that in *Cynias* " the dimension of the scale is the largest in the trunk, larger on the head, and the smallest on the tip of the fins." In *Squalus*, on the other hand, scales of the snout type are the largest and fin-marginals are next in size. Those on the tips of the fins are smallest, as in *Cynias*.

Steinhard's observations on the structure of the comb scales of the gill cavities have been verified by us. On the other hand, the stomodeal denticles described and depicted by Hertwig and by Steinhard do not seem to match exactly those found by us in the pharynx. We agree with Steinhard that the longitudinal element is much reduced or absent,

giving this scale a more delicate appearance in comparison with those of the skin. Two quite marked differences were not shown by him. The lateral elements of the basal plate project distinctly anterior in these denticles and the spine arises well toward the posterior end of this scale.

Steinhard (1903), Imms (1905), and Fahrenholz (1915) found stomodeal denticles to be missing from the roof of the mouth and pharynx but present on the lining of the gill arches and the entire covering of the floor of the mouth between the level of the ventral ends of the first functional gill slits and that of the corresponding parts of the fifth pair. Cook and Neal (1921), however, reported these scales to be present not only in the above-mentioned regions but also somewhat more anterior than the first slit and, more significant, on the roof of the pharynx in small numbers. We have been unable to find denticles on the roof of the mouth or pharynx medial to the region of the dorsal ends of the gill slits. They are, however, present along inner edges of the septa and in the floor of the pharynx. We agree with Steinhard and Imms that these scales are ordinarily found ventrally, only between the first and fifth slits. Our observations differ from those of others in that we have noted that stomodeal denticles are lacking in the lateral parts of the pharyngeal floor, except for occasional ones toward the posterior ends of these regions.

Steinhard (1903) reported that comb scales occur in the pharyngeal slits both internal and external to the lamellæ. A further study verifies this statement. Certain details may be added. The areas of comb scales external to the four demibranchs on the posterior walls of the slits are considerably wider than those external to the five demibranchs of the anterior walls. Comb scales are present in considerable numbers internal to the demibranchs of posterior walls but, except for the extreme ventral and dorsal ends, they are lacking internal to the demibranchs of anterior walls. There is, of course, no demibranch on the posterior wall of the fifth cleft. The external part of this wall bears no comb scales. The internal part has many of these scales and much of the area corresponding to the positions of demibranchs of other slits has scattered scales of this type.

The spiracular cleft does not have comb scales associated with it. There are, however, stomodeal denticles. These are numerous on the posterior wall, somewhat less abundant on the anterior wall, quite limited between these regions on the dorso-medial side, and absent from the ventro-lateral wall.

From our observations, we conclude that in the integument of *Squalus* of 54 to 62 cm. length there is a quite constant pattern of distribution of scaleless areas and of scales of various types. There are

at least four general types. Since three of these exist in two quite constant subtypes which do not seem to intergrade, the number of distinct types is probably about seven. In addition, there are two types which are almost certainly transitional. In the linings of the mouth, pharynx and gill slits there are two additional types—stomodeals and combs—as previously reported by Steinhard (1903) and others.

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HISTOCHEMISTRY OF THE OVARY OF FUNDULUS HETEROCLITUS WITH SPECIAL REFERENCE TO THE DIFFERENTIATING OOCYTES

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The process of differentiation in the oöcytes of many animals has been studied by histological and cytological methods. Although the general agreement in the morphological changes is very striking, the divergence in interpretation of the significance of the structures observed and the relationships between them is more so. Konopacki and Konopacka (1926), Konopacka (1935), and Guthrie (1925 and 1929) have emphasized the importance of a physiological viewpoint in such studies. The limitations of attempts to analyze the chemical contents of regions of a cell by means of their reactions with fixing or staining fluids are numerous, in spite of the fact that considerable security can be felt in some instances. With histochemical methods a somewhat different attack can be made on the question of the significance of changes in differentiating cells. Not only is the identification and specific localization of a variety of substances possible at different periods, but the use of quantitative standards of comparison gives data on the shifting concentration of materials, as Marza and Marza (1935) and Marza (1935) have shown in the hen's egg. The present collaboration was undertaken in order to provide histochemical data on a form in which cytological studies had been made, with the expectation that the sequences of differentiation in the oöcytes would be revealed more clearly.

Observations have been made on all parts of the ovary—stroma, interstitial cells, follicular theca, follicular epithelium, zona radiata, and oöcytes in all stages of differentiation. For purposes of reference in the descriptions the differentiating oöcytes are placed in two main groups (Fig. 1). Those in the first part of the growth-period (Period 1) have increasing amounts of cytoplasm, with cytosomes that may range up to 300 microns in diameter, but no yolk vesicles (Figs. 8, 12, and 16).

[1] Dr. and Mrs. Marza have made all of the histochemical analyses recorded in this paper. Their report has been prepared for publication by Mary J. Guthrie, who provided the specimens, through the Supply Department of the Marine Biological Laboratory, at Woods Hole, Massachusetts, U. S. A., and who has correlated the histochemical observations with cytological observations on samples from the same and many other specimens.

Period 2 begins with the appearance of vesicles (proteinaceous yolk vesicles of Guthrie, 1928) in the oöcytes and includes the remainder of the period of differentiation. During stage A_1 of the second period there are few yolk vesicles and the diameter of the cell may be as great as 400 microns (Figs. 16, 17, and 18). The increase in number of yolk vesicles distinguishes stage A_2, at the end of which only a narrow zone

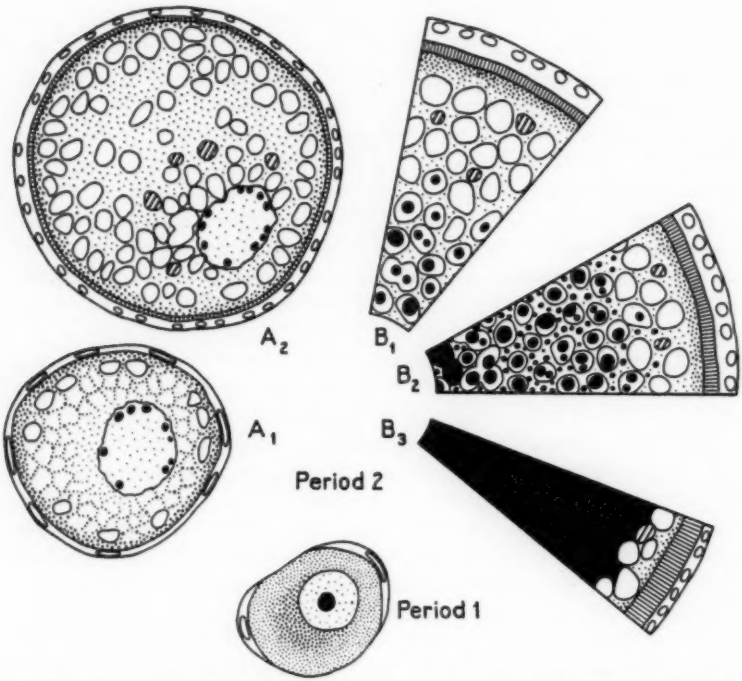


FIG. 1. Diagram showing the stages in the growth-period of oöcytes in *Fundulus* which are referred to throughout the text. In the cytosome the circles which are empty represent the vesicles in which yolk may appear later; circles which are lined represent fat deposits; and circles which are solid represent either intra- or extravesicular yolk.

of peripheral cytoplasm is free of vesicles and cells may be 600 microns in diameter (Fig. 14). Between the vesicles the internal cytoplasm is seen as a reticulum. The zona radiata appears at this stage, and the nucleus of the oöcyte is increasingly eccentric. The beginning of stage B_1 is marked by the initial appearance of globules of yolk (intravesicular yolk) within the vesicles (Figs. 10 and 13). During stage

B_2 globules of extravesicular yolk begin to appear directly in the cytoplasm between the vesicles, and the diameter of the oöcyte may reach 1,000 microns (Fig. 15). The globules of yolk that appear within the so-called proteinaceous yolk vesicles, as well as those which appear between the vesicles, constitute the fatty yolk of Guthrie (1928). As the oöcyte continues to grow, intravesicular and extravesicular yolk increases in amount. The yolk globules deposited within and without the original large vesicles become confluent and indistinguishable (Fig. 13). Toward the end of the growth-period (stage B_3), a continuous mass of yolk is found surrounded by a cortical layer of cytoplasm (Figs. 11, 12, and 13) in which the germinal vesicle is located. Primary oöcytes may reach 1,600 microns in diameter.

This report includes observations on the variations in amount and distribution of iron and potassium, on the plasmal and nucleal reactions, and on the localization and relations of the acid proteins. Other analyses are in progress.

Determination of Iron

Iron occurs in fish eggs in the ichthulin molecule which, like vitellin, contains a hematogen (Walter, 1891, in the carp). A histochemical study of iron has been made by Smiechowski (1892), Wassermann (1910), Marza, Marza, and Chiosa (1932), and Marza (1935) in the hen's egg at various stages in its growth. In *Fundulus heteroclitus* we have studied the localization and changes in content of iron in the oöcytes during the period of growth and yolk-formation.

The ovaries used were from fish collected at Woods Hole, Massachusetts, on June 27 and September 11, 1934. Fixation was in 96 per cent alcohol, and paraffin sections 10 microns in thickness were studied. Since it seemed probable that both inorganic and organic iron were present in eggs (*cf.* Warburg, 1914, on eggs of *Strongylocentrotus*), methods for detecting inorganic and total iron content were used. Macallum's (1912) ammonium-sulfide method and Liesegang's (1923) potassium-ferrocyanide method were employed to test for inorganic iron, while Policard's (1924) microincineration method made possible the identification and localization of the total iron content.

With Macallum's method certain granules in the stroma, follicle, and oöcyte become dark brown, which presumably is evidence of their inorganic iron content. However, some of these dark brown granules are apparently pigment since they are seen in similar locations after the use of Liesegang's method, with which the presence of inorganic iron is indicated by a homogeneous blue staining of certain regions. It ap-

pears, therefore, that Macallum's method is not entirely trustworthy for this material. Tests for inorganic iron have not been considered positive unless the results with the two methods were in agreement. After

TABLE I

Results of tests for inorganic iron and total iron content. Four hundred and twenty-eight oöcytes in 12 ovaries were examined. A (?) indicates that a result is considered unreliable for reasons stated in the text.

Region	Methods		
	Macallum	Liesegang	Policard
Interstitial cells	+	+	+
Stroma	+ (?)	−	−
Theca			
Period 2 A	+	+	− (?)
Period 2 B	+	+	− (?)
Follicular epithelium			
Period 1	−	−	−
Period 2 A	+	+	+
Period 2 B	+	+	+
Zona radiata			
Period 2 A	+	+	−
Period 2 B	+	+	−
Oöcyte cytoplasm			
Period 1	−	−	−
Peripheral region			
Period 2 A	+	+	− (?)
Period 2 B	+	+	− (?)
Internal region			
Period 2 A	+	+	+
Period 2 B	+	+	− (?)
Yolk			
Intravesicular	+	+	+
Extravesicular	−	−	+
Oöcyte nucleus			
Chromatin	−	−	−
Nucleoli	+ (?)	−	−

the incineration of sections according to the method of Policard the various parts of the ovary are recognizable. Of the mineral residues only the oxides of iron are colored orange or red; others are white or gray. The detection of iron and its localization within the cells are

thus possible. Microincinerated sections were examined against a dark field with a Greenough microscope and, also, by means of a Reichert oblique illuminator.

Inorganic iron is most abundant in the interstitial cells, some of which give a more intense reaction than others, and is rarely seen in the stroma. The nucleus of the growing oöcyte does not contain iron at any stage. During the first part of the growth-period iron has not been demonstrated either in the cells of the follicle or in the cytoplasm of the oöcyte. However, in the second period inorganic iron is sometimes seen in the follicle cells and in the zona radiata but not throughout the entire circumference of any one egg. Within the oöcyte at this stage a positive inorganic iron test of weak intensity is sometimes obtained in the peripheral layer of cytoplasm, as well as in the internal cytoplasm between the yolk vesicles. In the intravesicular yolk the inorganic iron test is sometimes positive, but in the extravesicular yolk inorganic iron has not been detected. After microincineration the fully differentiated yolk is seen to contain iron, which must have been in organic combination (Fig. 11). The summary of tests for iron is given in Table I.

It should also be recorded that white ash (probably calcium) is abundant in the cytoplasm of oöcytes in Period 1 (Fig. 8). These oöcytes differ strikingly from those of the hen, which do not contain ash at a comparable stage. White ash also occurs in both the peripheral and internal cytoplasmic regions during Period 2 in oöcytes of *Fundulus* (Fig. 9). This white ash may obscure the red where it is present in small amounts and render the negative microincineration results for iron unreliable for certain regions. As the intravesicular yolk appears a gray ash remains after microincineration (Fig. 10). The nucleoli of the fish egg are very rich in white ash, and the chromatin in gray ash.

Inorganic iron apparently passes through the follicle and zona radiata of the oöcyte during the period of yolk deposition and is found in the cytoplasm and in the yolk forming within the vesicles. During the final differentiation of yolk the accumulated iron must be combined in organic form, probably in the hematogen of ichthulin. The finding of iron in both inorganic and organic form may account for certain discrepancies in previous reports.

Quantitative Determination of Potassium

The quantity of potassium exceeds the combined amounts of calcium, sodium, and magnesium in ripe fish eggs. Bialaszewicz (1929) has shown that high potassium content is characteristic of egg cytoplasm

of both invertebrates and vertebrates. Seventy-four to 92 per cent of all the diffusible bases in egg cytoplasm is potassium. Chemically the potassium content of a number of ripe and fertilized fish eggs has been determined (Needham, 1931, p. 356), but the eggs of *Fundulus* have not been studied from this point of view. A histochemical study of potassium has not been made on any fish egg, nor have studies been made on any differentiating oöcytes.

The method used was that of Macallum (1905) as modified by Marza and Chiosa in 1934 and 1935. By means of a comparison eye-

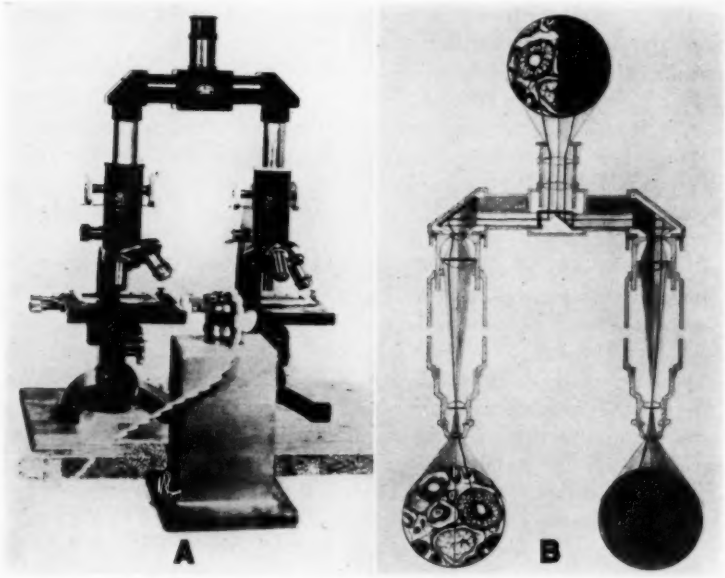


FIG. 2. Use of comparison eyepiece in quantitative histochemical observations. *A*, arrangement of microscopes and light; *B*, diagram of preparation of ovary (left, below), standard slide (right, below), and field of vision (center, above).

piece and a series of standards made of agar-agar and containing concentrations of cobalt sulphide calculated as potassium equivalents, it is possible to determine the quantity of potassium in specific regions of the ovaries (Fig. 2).[2] Ovaries from twelve fish were fixed in 96 per cent alcohol and sectioned in paraffin at 10 microns. One hundred and thirty-six oöcytes of Period 1, 63 of Period 2A, and 72 of Period 2B

[2] Marza and Chiosa (1935) have given the technique for the preparation and use of the standard slides.

have been studied. Within these stages of differentiation the oöcytes observed have been sorted according to actual size attained.

The smallest oöcytes contain more potassium in their cytosomes than do larger ones (Figs. 3 and 12, and Table II). Since these cells, which are from 20 to 50 microns in diameter, do not have a continuous follicular layer the concentration of potassium is not conditioned by selective permeability of the follicle (Fig. 1). The nucleoli of such young oöcytes are very rich in potassium but the chromatin contains little.

TABLE II

Results of tests for potassium, indicated as average percentages. Two hundred and seventy-one oöcytes in 12 ovaries were examined.

Region	Percentage K	Region	Percentage K
Stroma		Oöcyte cytoplasm	
Cytoplasm	0.065	Period 1	0.160
Nucleus	0.130	Peripheral region	
		Period 2 A	0.087
Theca		Period 2 B	0.084
Cytoplasm		Internal region	
Period 2 A and B	0.032	Period 2 A	0.087
Nucleus		Period 2 B	0.109
Period 2 A and B	0.087		
Follicular epithelium		Yolk	
Cytoplasm		Intravesicular	0.108
Period 1	—	Extravesicular	0.075
Period 2 A	0.066		
Period 2 B	0.057	Oöcyte nucleus	
Nucleus		Chromatin	
Period 1	—	Period 1	0.098
Period 2 A	0.109	Period 2 A	0.049
Period 2 B	0.116	Period 2 B	0.065
		Nucleoli	
Zona radiata		Period 1	0.151
Period 2 A	0.054	Period 2 A	0.125
Period 2 B	0.057	Period 2 B	0.130

During the second period the theca is somewhat more conspicuous, and the concentration of potassium is not the same in it as in the cells of the stroma (Table II). In the follicle cells the nuclei are richer in potassium than are the cytosomes. No concentration of potassium occurs in the zona radiata. With the appearance of vesicles in the oöcyte (Period 2 A_1) the concentration of potassium in the cytoplasm continues to decrease (Fig. 3). The penetration of potassium does not keep pace with the rate of cytoplasmic increase in the oöcyte. This de-

crease is noticeable, also, in the chromatin and nucleoli. During the early stages of yolk deposition the percentage of potassium in the cyto-

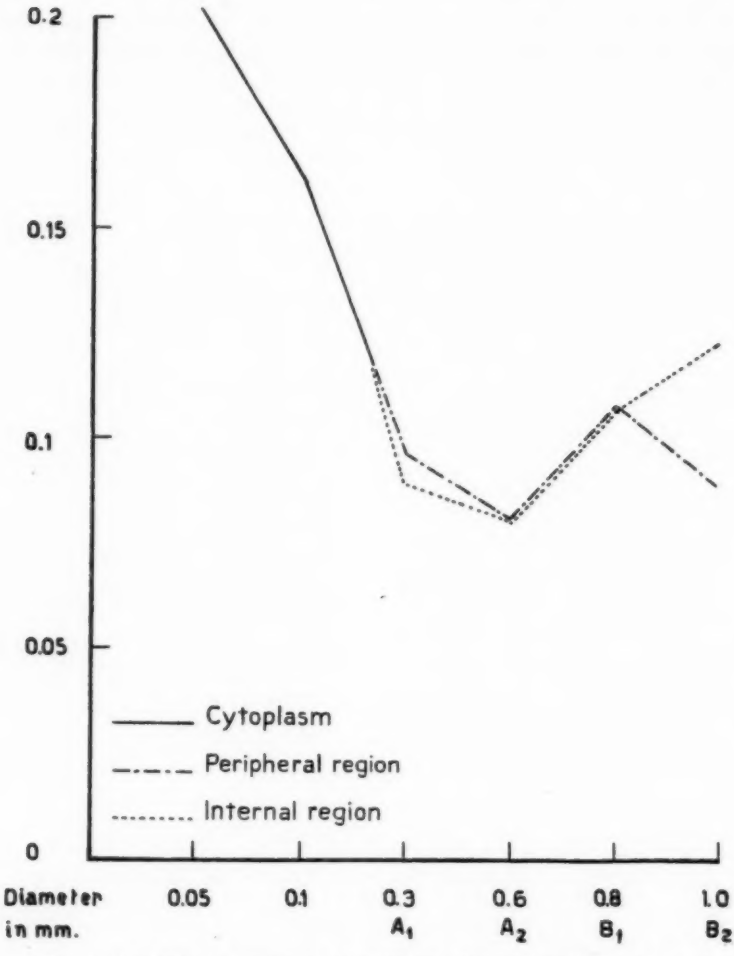


FIG. 3. Variation in percentage of potassium, shown on vertical axis, in cytoplasm of oöcytes during the growth-period.

plasm begins to rise, especially in the internal region. Yolk contains a high percentage of potassium which again falls off as the oöcyte grows

(Fig. 12). A similar shift in proportion of potassium during yolk-formation has been observed in the hen's egg in the ground substance of both white and yellow yolk-spheres.

The fact that the theca and follicular epithelium vary so little with respect to potassium content throughout the growth-period is different from the findings in the ovary of the hen (Marza and Chiosa, 1936). There, toward the end of yolk-formation, the percentage of potassium increases in the follicle cells. In the ovary of the fish there appears to be little increased concentration, or temporary storage, in the follicle cells of substances utilized in the formation of yolk. However, there is some indication that the follicle cells determine the rate of penetration of potassium which decreases in amount in the cytoplasm of the oöcyte following the association of follicle cells. The follicular epithelium apparently undergoes changes in permeability. It seems to be more permeable to potassium during stages A_2 and B_1 and only slightly permeable at later stages. That the result may be conditioned by an altered rate of entrance of other materials is also a possibility.

Plasmal Reaction

In 1924 two color reactions were reported for cells after the application of Schiff's sulfurous-fuchsin reagent. The first or plasmal reaction was observed in the cytosome, and the nuclei were uncolored except when the reagent was applied for a long period (Feulgen and Voit, 1924). If the sections were hydrolyzed with hydrochloric acid before being treated with Schiff's reagent, the color reaction was in the nucleus; this was called the nucleal reaction (Feulgen and Rossenbeck, 1924).

The names given to these reactions indicate that aldehydes in the cytoplasm or chromatin were believed to be responsible for the appearance of the color. Feulgen and Voit (1924) pointed out that the plasmal reaction was related to the presence of fat in the cytoplasm. If the tissue is dehydrated before sectioning the reaction is usually negative. Verne (1929) demonstrated that only unsaturated fats in the process of oxidation gave a positive plasmal reaction. However, fatty substances are not the only ones to react positively. Feulgen and Voit (1924), Voss (1929), and Marza and Marza (1934) have reported an intense plasmal reaction in the elastic elements of large blood vessels, and it occurs in tissue dehydrated after fixation. Many who use this method of Feulgen consider it to be specific for aldehydes. Feulgen and co-workers pointed out that while Schiff's reagent reacted with alkalis (a red color) and bromine they considered it to be specific for

aldehydes in cells. Lison (1932) found that the color of the reaction with various aldehydes varied from deep violet to blood red. He concluded, therefore, that similar color reactions by substances other than aldehydes should be considered as positive. With this criterion alkalis, aliphatic ketones, certain unsaturated compounds (such as oleic acid),

TABLE III

Results of plasmal and nucleal reactions, indicated as averages of color intensity; 0.3 is the lowest positive reading.

Region	Plasmal reaction	Nucleal reaction
Stroma		
Cytoplasm	–	–
Nucleus	–	1.25
Theca		
Cytoplasm	–	–
Nucleus	–	1.25
Follicular epithelium		
Cytoplasm	–	–
Chromatin	–	1.25
Nucleoli	–	1.25
Nucleoplasm	–	0.3
Zona radiata	0.3	0.3
Oöcyte cytoplasm		
Period 1	0.3	0.4
Peripheral region		
Period 2 A and B	0.3	0.3
Internal region		
Period 2 A and B	0.3	0.3
Yolk		
Intravesicular	0.75	0.75
Extravesicular	0.6	0.5
Oöcyte nucleus		
Period 1	–	–
Period 2 A	–	–

weak salts of strong bases (such as acetates and phosphates), some amino oxides, and certain catalytic oxidizing systems gave positive color tests. Even though certain of these substances occur not at all or in very minute quantity in normal cells, the presence of alkalis, phosphates, ketones, and oxidases makes it clear that a positive plasmal reaction cannot be considered specific for aldehydes or fats.

Voss (1927) first studied the plasmal reaction during the growth-period of oöcytes, using ovaries of amphibians. Hibbard (1928) and Brachet (1929) employed it with amphibian ovaries, and Marza and

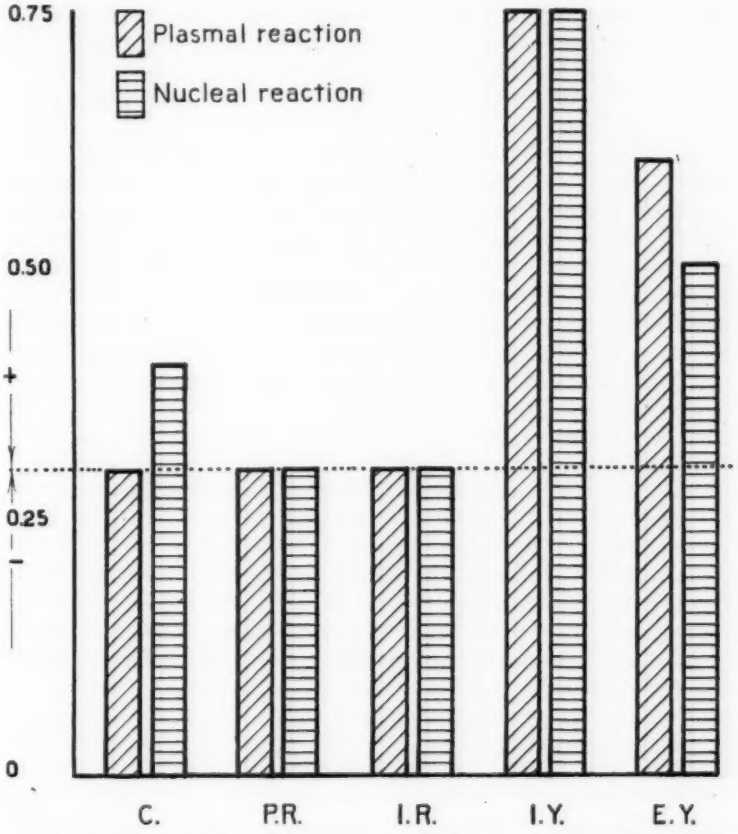


FIG. 4. Plasmal and nucleal reactions in the oöcytes. *C*, cytoplasm of oöcytes of Period 1; *P. R.*, peripheral region of cytoplasm of oöcytes of Period 2; *I. R.*, internal region of cytoplasm of oöcytes of Period 2; *I. Y.*, intravesicular yolk; and *E. Y.*, extravesicular yolk. The numbers on the vertical axis refer to the comparison color scale.

Marza (1934) with the hen's ovary. For the reaction in *Fundulus*, twelve ovaries were fixed in sublimate-acetic as recommended by Feulgen. Frozen sections were used in order not to lose the fatty sub-

stances during dehydration. The sulfurous-fuchsin reagent was prepared according to Wermel (1927),[3] and the short technique was followed: thirty minutes in the reagent, rapid washing in three jars of water turbid with sulfur dioxide, washing in distilled water, dehydration, and mounting. In order to evaluate the reaction qualitatively a scale of color intensity was employed. An intensity designated as 0.3 indicates the least positive reaction and below that value the coloring is the result of adsorption of the reagent; 3.0 is an extremely intense positive reaction.

A slightly positive plasmal reaction may be obtained in the cytoplasm of oöcytes of Period 1 (Table III and Fig. 4). In this material the so-called yolk-nucleus, as described by Hibbard (1928) in *Discoglossus* and Marza and Marza (1934) in the hen, is not distinguishable. The reaction of the nucleus of the oöcyte is negative at all times except for an occasional faint reaction in a nucleolus. No reaction is observed in the ovarian stroma, the theca, or the follicular epithelium at any stage. The interstitial cells give an intense positive reaction.

During the second period the zona radiata varies in reaction, apparently depending on the material present in its canals at any particular time; it is positive in about half the cases. In the peripheral and internal cytoplasm of the oöcyte the reaction is variable with an average on the positive side. Yolk forming within the vesicles gives a more positive reaction than that formed directly in the cytoplasm. Since the reaction is the same in sections dehydrated in alcohol before being treated with the Schiff's reagent, the substance responsible cannot be fat. The difference in degree of reaction in the intravesicular and extravesicular yolk parallels the difference in potassium content. It seems possible that the plasmal reaction in the yolk is conditioned by the presence of the alkali.

Nucleal Reaction

The nucleal reaction has been studied in the eggs of numerous invertebrates and some vertebrates. Contrary to the usual positive reaction in the nuclei of cells, the nucleal reaction becomes negative in the nuclei of oöcytes early in the growth-period and remains negative until the period of diakinesis (Koch, 1925; Voss, 1927; Hibbard, 1928; Ludford, 1928; Brachet, 1929; Gresson, 1930; Mukerji, 1930; Bauer, 1932;

[3] Dissolve 1 gram of basic fuchsin in 200 cc. of boiling distilled water and filter. (Grübler's gelblich fuchsin was used.) Add 20 cc. of normal hydrochloric acid. When the solution is cool add 2 grams sodium bisulfite and stir for several minutes. After 2 hours add 0.2 cc. acetaldehyde; the solution becomes intensely red. After 45 minutes again add 20 cc. of normal hydrochloric acid and 1 gram of sodium bisulfite. Stir the solution for 15 minutes and set aside until the fuchsin is decolorized. The pale amber reagent should be kept dark and cool.

Marza and Marza, 1934). This peculiar situation is characteristic of only female germ cells, and the nucleal reaction is positive during the entire course of spermatogenesis.

Ovaries of *Fundulus* were fixed in sublimate-acetic, and the Feulgen-Rossenbeck technique was followed on paraffin sections. The color scale used for the plasmal reaction records was again employed.

The cytoplasm of cells of the stroma and of the follicular epithelium has a negative nucleal reaction (Table III). The nuclear membrane and chromatin, but not the nucleoplasm, of cells of the stroma give positive tests. In the nuclei of the follicular epithelium the reaction is negative in the membrane, faintly positive in the nucleoplasm, and equally strong in the chromatin and in the nucleoli. These reactions do not change conspicuously during the course of differentiation.

There is a positive reaction in the cytoplasm of the oöcyte during the first period of differentiation which is similar to the reaction without hydrolysis—the plasmal reaction (Fig. 4). It seems likely that this is to be explained on the basis of the potassium content. Even in the smallest oöcytes the nucleal reaction is negative in all parts of the nucleus.

During the second period of differentiation the zona radiata has a faintly positive reaction in stage B. The cytoplasm at the periphery of the oöcyte and between the yolk globules gives a faintly positive reaction. As with the plasmal tests, the yolk gives a positive nucleal reaction of similar intensity. Since hydrolysis does not alter the reaction, it is apparent that it is not always conditioned by fat, and that it may be attributed to the potassium content. In the oöcyte of the hen Marza and Marza (1934) made further tests that led them to the hypothesis that the positive plasmal and nucleal reactions arise from the presence of potassium. They found in the yolk of the hen's egg that the plasmal and nucleal reactions were localized in the centers of the globules. When the nucleoprotein test (method of Unna, 1921) was positive the localization was peripheral. The reaction for oxidases was negative. Prolonged extraction with ether and chloroform did not alter the reaction, which eliminated fats as the source of aldehyde. There was no augmentation of the reaction after treatment with alcohol; it had been suggested that alcohol might be adsorbed by the yolk and partially changed to aldehyde.

The nucleal reaction is negative in nuclei of all oöcytes during the second period in *Fundulus*, and no observations were made on the time of shift of the reaction before meiosis. There is no evidence upon which to offer an explanation of this negative test. Koch (1925) and Brachet (1933) attributed it to a chemical change in the nucleic acid

during the growth-period, while Feulgen (1926) and Ludford (1928) considered it to be the effect of dispersion or dilution of thymonucleic acid as the nucleus of the oöcyte increased in volume.

Acid Proteins

In order to study changes in the proteins during differentiation of the oöcyte the method of Unna (1921) for acid proteins was used. This procedure enables one to distinguish albumins, globulins, and nucleoproteins. It is based on differences in color reaction and solubility between the groups. Tissues are fixed in 96 per cent alcohol, sectioned in paraffin, and stained for twenty minutes in the Pappenheim-Unna reagent.[4] Albumins and globulins are stained red by the pyronin, while the nucleoproteins are stained blue-green with the methyl green. To separate the albumins and globulins advantage is taken of their specific solubilities. Albumins and pseudoglobulins are soluble in distilled water while globulins are not. Both albumins and globulins are soluble in salt solutions. By comparison of sections stained before and after chromolysis in water and in salt solution the location and relative proportions of acid proteins of the three groups can be determined.

Sections of the ovary of *Fundulus* were divided into three series. One series was stained directly. In such sections all acid proteins were shown. A second series was placed in sterile distilled water in an oven at 39° C. for twenty-four hours before staining. This was the first chromolysis. The difference in reaction with pyronine between these sections and those of the first series was conditioned by the loss of albumins (*cf.* Figs. 16 and 18). A third series was placed in a sterile 2 per cent solution of sodium chloride in an oven at 39° C. for twenty-four to forty-eight hours before staining. This was the second chromolysis which removed both albumins and globulins. If nucleoproteins were present in such sections they would then be stained with the methyl green (*cf.* Fig. 17 with Figs. 16 and 18). To obtain results subject to comparison, sections of the three series were handled simultaneously by an arrangement of forceps during the staining and dehydration. The latter process had to be completed rapidly. Results were read on a standard color scale in which 0.3 was the least positive reaction and 4.0 the most intense. Two hundred and sixteen oöcytes have been studied in sections from twelve ovaries.

Two varieties of interstitial cells are demonstrated with Unna's technique. Those which are pigmented, occur in masses, and contain

[4] The Pappenheim-Unna reagent used consisted of 0.15 gram of Grübler's methyl green (from which admixed methyl violet was removed by shaking the crystals in chloroform), 0.25 gram of pyronine, 2.5 cc. of 96 per cent alcohol, and 100 cc. of 0.5 per cent phenol.

iron do not color with the stains used. Cells of the other type have variable shapes, with pseudopodia-like histiocytes or simple elongations like fibroblasts, or may be oval with eccentric nuclei somewhat similar

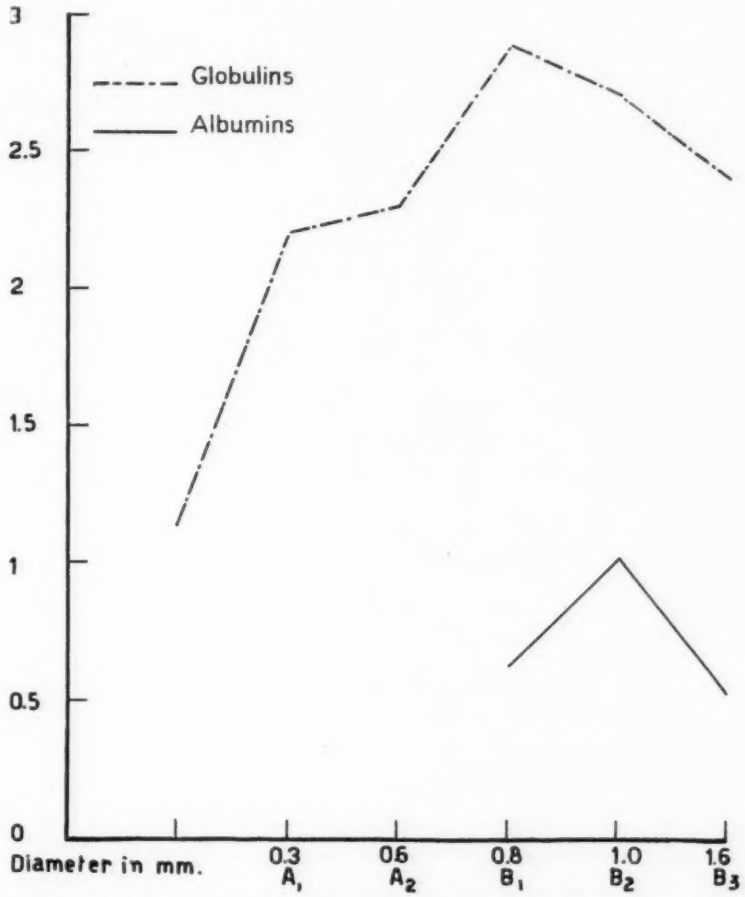


FIG. 5. Variation in acid proteins in the cytoplasm of follicle cells. The numbers on the vertical axis refer to the comparison color scale.

to plasmocytes. Such cells have been described in the ovarian stroma of the hen by Goodale (1919), Nonidez (1921), Marza (1934), and Marza and Golaescu (1935); the analogies are not completely clear.

These cells in *Fundulus* are found in the endothelium of the blood vessels, isolated in the stroma, in the tunica albuginea, and, rarely, within the masses of pigmented cells. The cytoplasm of the non-pigmented interstitial cells stains intensely with the pyronine.

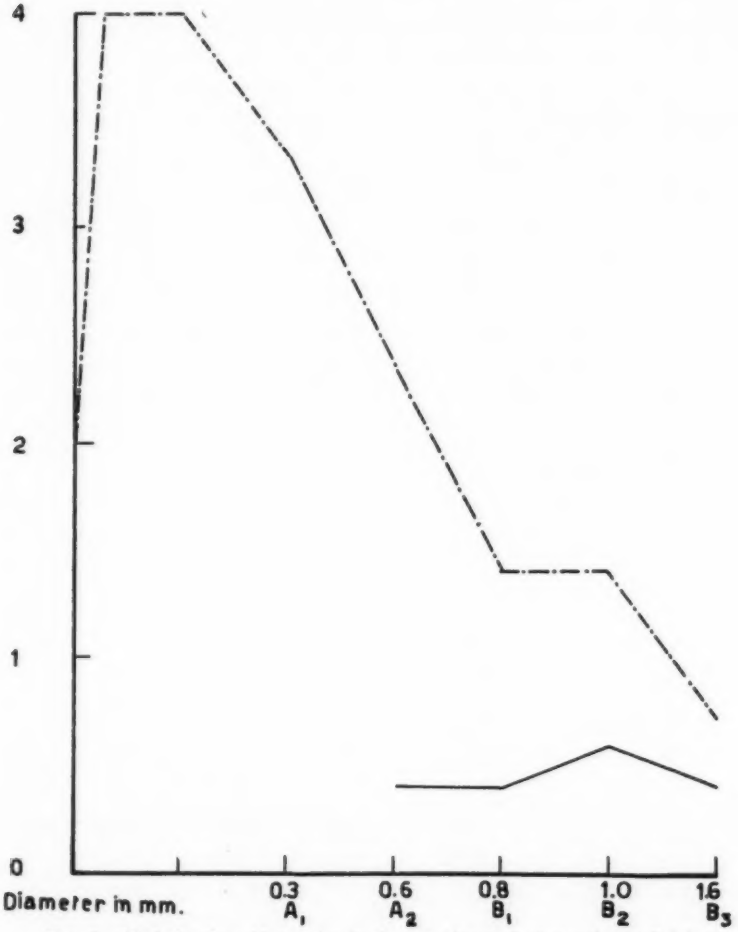


FIG. 6. Variation in acid proteins in the cytoplasm of oöcytes in Period 1 and in the peripheral region of cytoplasm in Period 2. Designations as in Fig. 5.

Only a small quantity of irregularly localized globulin is found in the cytoplasm of cells of the ovarian stroma, and neither globulins nor

albumins occur in the nuclei which are, however, rich in nucleoproteins (Table IV). Both globulins and albumins are found in the cytoplasm of thecal cells during Period 2A; the amount is somewhat reduced

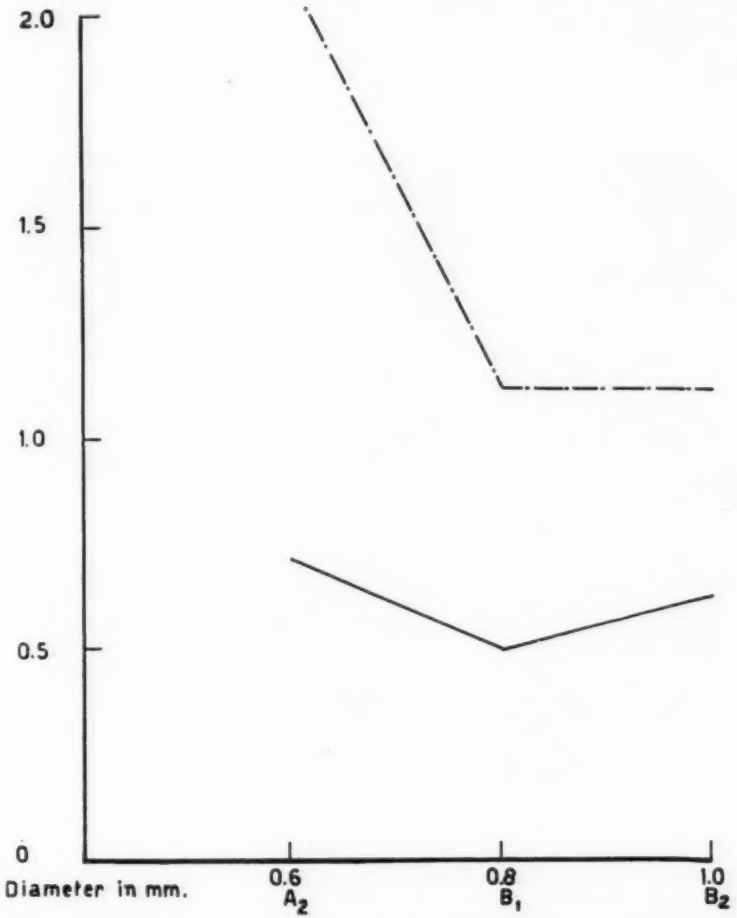
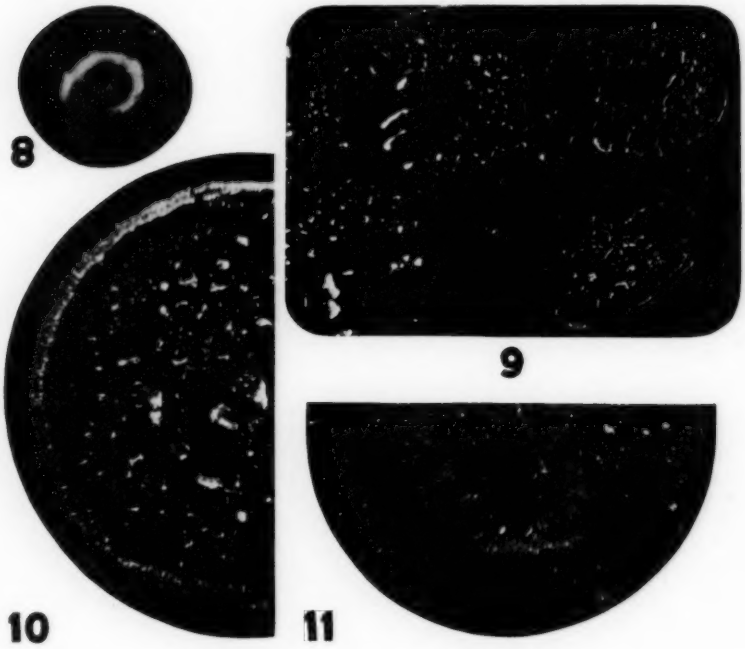


FIG. 7. Variations in acid proteins in the internal regions of cytoplasm in oöcytes of Period 2. Designations as in Fig. 5.

during Period 2 B. The nuclei of thecal cells are rich in nucleoproteins. Concentrated masses of globulins are seen localized along the length of the cytosomes of follicle cells during Period 1 (Fig. 5), but the ground

cytoplasm of follicle cells gives only a slight reaction (0.5). The amount of globulins in the follicle is increased conspicuously during Period 2, and albumins appear in stage 2B. These albumins may occur because of the splitting of globulins in the follicle as penetration into the oöcyte begins. Globulins appear in the chromatin during stage 2 B and are found, together with nucleoproteins, in the nucleoli throughout the growth-period. No acid proteins have been detected in the zona radiata at any time.



EXPLANATION OF PLATE I

Microphotographs

FIG. 8. Small oöcyte (Period 1) after microincineration, showing much white ash in cytosome and some in nucleus. × 150.

FIG. 9. Oöcytes of Period 2 A after microincineration. Ash is visible in the cytoplasm but not in the vesicles. × 30.

FIG. 10. Oöcyte of Period 2 B_1 after microincineration. White ash occurs in the region of the cytoplasm and gray ash in the intravesicular yolk which is present. The follicle appears two-layered because of the plane of the section. × 56.

FIG. 11. Oöcyte of Period 2 B_3 after microincineration. The homogeneous central mass of yolk is somewhat shrunken from the peripheral cytoplasm and its ash content is visible. × 30.

TABLE IV

Results of tests for acid proteins, indicated as averages of color intensity; 0.3 is the lowest positive reading. Where a range is given it indicates a shift in content between the smaller and larger cells of a given period.

Regions	Albumins	Globulins	Nucleoproteins
Stroma			
Cytoplasm	—	0.8	—
Chromatin	—	—	2.5
Nucleoli	—	—	3.0
Theca			
Cytoplasm			
Period 2 A	0.4	0.6	—
Period 2 B	—	0.6	—
Chromatin			
Period 2 A and B	—	—	2.0
Follicular epithelium			
Cytoplasm			
Period 1	—	1.1	—
Period 2 A_1–A_2	—	2.2–2.3	—
Period 2 B_1	0.6	2.9	—
Period 2 B_2–B_3	1.0–0.5	2.7–2.4	—
Chromatin			
Period 1	—	—	2.5
Period 2 A	—	—	3.0
Period 2 B	—	3.0	3.0
Nucleoli			
Periods 1 and 2	—	3.0	3.0
Oöcyte cytoplasm			
Period 1	—	2.0–4.0	—
Peripheral region			
Period 2 A_1	—	3.4	—
Period 2 A_2	0.4	2.4	—
Period 2 B_1	0.4	1.4	—
Period 2 B_2–B_3	0.6–0.4	1.4–0.7	—
Internal region			
Period 2 A_2	0.7	2.4	—
Period 2 B_1	0.5	1.1	—
Period 2 B_2	0.6	1.1	—
Yolk			
Intravesicular			
Period 2 B_1	—	0.75	0.5
Period 2 B_2	—	0.5	0.5
Extravesicular			
Period 2 B_2	—	—	0.5
Oöcyte nucleus			
Chromatin			
Periods 1 and 2	0.3	0.5	—
Nucleoli			
Periods 1 and 2	—	3.5	—

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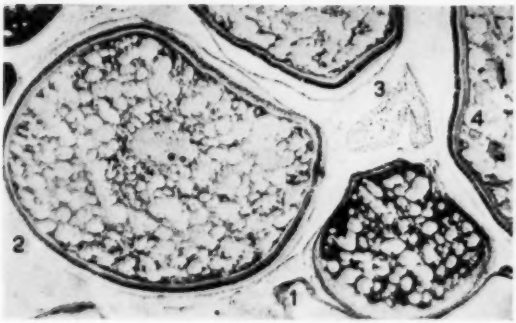

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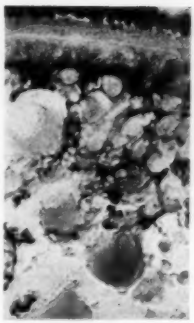

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EXPLANATION OF PLATE II

Microphotographs

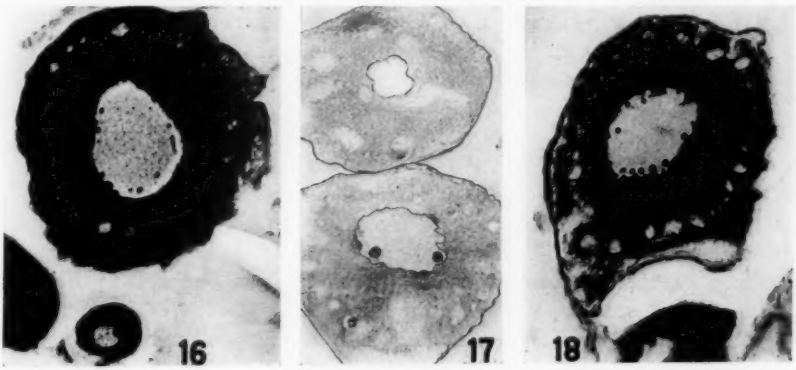
FIG. 12. Group of oöcytes, showing variation in potassium content; method of Macallum. × 41. Oöcyte 1 is in Period 1 and has a high potassium content. Oöcyte 2 is in Period 2 B_1; 3 is in Period 2 B_2; and 4 is in Period 2 B_3 (4 is about 1,200 microns in diameter). Note the decrease in potassium content in the yolk in oöcytes 3 and 4.

FIG. 13. Group of oöcytes showing variation in total acid proteins; method of Unna, without chromolysis. × 17. Oöcyte 1 is in Period 2 A_2 and gives an intense reaction in the cytoplasm; oöcyte 2 is in Period 2 B_1; 3 is in Period 2 B_2; and 4 is in Period 2 B_3 with a central homogeneous mass of yolk (4 is about 1,200 microns in diameter). Note shift in reaction in yolk.

FIG. 14. Group of oöcytes showing variation in total acid proteins; method of Unna, without chromolysis. × 41. Oöcytes 1, 2, and 3 are in Period 2 A_2 with numerous vesicles; and 4 is in Period 2 B_3. Note shift in reaction in cytoplasm.

FIG. 15. Portion of oöcyte early in Period 2 B_3 showing variation in acid proteins; method of Unna, without chromolysis. × 204. Note larger heterogeneous vesicles containing some intravesicular yolk and the much smaller globules of extravesicular yolk.

Acid proteins with a globulin reaction are conspicuous in the cytoplasm of the smallest oöcytes and reach a very high concentration by the end of Period 1 (Fig. 6). They are uniformly distributed in the cytosome (Figs. 16 and 18). No albumins or nucleoproteins are observed at this stage (Table IV). As vesicles appear and increase in number the peripheral layer of cytoplasm has a decreasing amount of globulins (Figs. 6, 13, 14, and 15). Again we see in the oöcyte of *Fundulus* during the first period a conspicuous accumulation of sub-



EXPLANATION OF PLATE III

Microphotographs

FIG. 16. Oöcytes showing intense acid protein reaction; method of Unna, without chromolysis. × 140. Smaller oöcyte is in Period 1, larger is in Period 2 A_1.

FIG. 17. Oöcytes in Period 2 A_1, showing the absence of globulins; method of Unna, after chromolysis in salt solution. × 140. The failure to stain with methyl green indicates the absence of nucleoproteins.

FIG. 18. Oöcytes showing globulin content; method of Unna, after chromolysis in water. × 140. Smaller oöcyte, of which only a part is seen, is in Period 1; larger is in Period 2 A_1.

stances before the actual synthesis of yolk begins. Albumins appear toward the end of stage 2 A and are found in small quantities until the end of yolk-formation (Fig. 6). In general there is less acid protein in the internal cytoplasm, but the albumins are somewhat more abundant (Fig. 7). It would appear as if proteins adjacent to the places of yolk deposition were chiefly in the form of simple albumins. However, in the yolk deposited within the vesicles some globulins are found but no albumins, while in extravesicular yolk neither can be demonstrated (Fig. 15). The synthesis of simple acid proteins into ichthulin and its combination with other groupings is apparently complete and not de-

stroyed by the method of treatment. This differs from the findings in the hen's egg where Marza (1935) found that globulins and pseudoglobulins were detectable in the fully differentiated yolk. The yolk in *Fundulus* contains an appreciable amount of nucleoprotein, much more than the yolk of the hen's egg. Konopacka (1935) has reported the presence of nucleoproteins in the yolk of two fresh-water fishes; she has also identified a mucoprotein in the vesicles of Period 2A.

The presence of nucleoproteins in eggs has stimulated considerable discussion. From an analysis of the ichthulin of various fish eggs Walter (1891), Hammarsten (1905), and Linnert (1909) concluded that there was no nucleic acid present. However, König and Grossfeld (1913) and others have isolated small quantities of purine bases or nucleic acids from the whole eggs of several fishes. The eggs of animals which develop in an aquatic environment contain the constituents of nucleoproteins, while the eggs of terrestrial animals are very poor in them. Needham (1931) has considered these facts and their implications. The histochemical method of Unna (1921) reveals the presence of nucleoprotein in the growing oöcyte of *Fundulus* which confirms the chemical findings in other teleosts. It may be that the positive Feulgen-Rossenbeck (1924) reaction in yolk is conditioned in part by nucleoprotein, but the presence of potassium in the same region confuses the interpretation.

The nucleus of the oöcyte does not give a nucleoprotein reaction at any time (Table IV). Both globulins and albumins occur in the chromatin masses. Nucleoli are rich in acid proteins, but only globulins are present. In the largest nucleoli the globulin reaction at the centers is 2.0 and at the peripheries, 4.0. Smaller nucleoli give a homogeneous reaction like the cortices of the larger ones. The absence of nucleoprotein has been considered in the discussion of the nucleal reaction. Unna's method does not provide data on which a decision can be made concerning the reason for the absence of the nucleal reaction in the nucleus of growing oöcytes.

The Follicular Epithelium and Zona Radiata

It is very striking that the concentrations of the substances recorded in this paper shift conspicuously in the oöcytes during the growth-period. This might be a result of changing resources in the blood stream, or of a selective action of the follicle and zona, or, in some cases, of synthetic activity within the oöcyte in which combination altered the quantity of a substance free to react with the testing reagent.

Since variations in height of epithelial cells have been taken in many cases to indicate degree of metabolic activity, measurements of the width of the follicle were made. At the same time the zona radiata

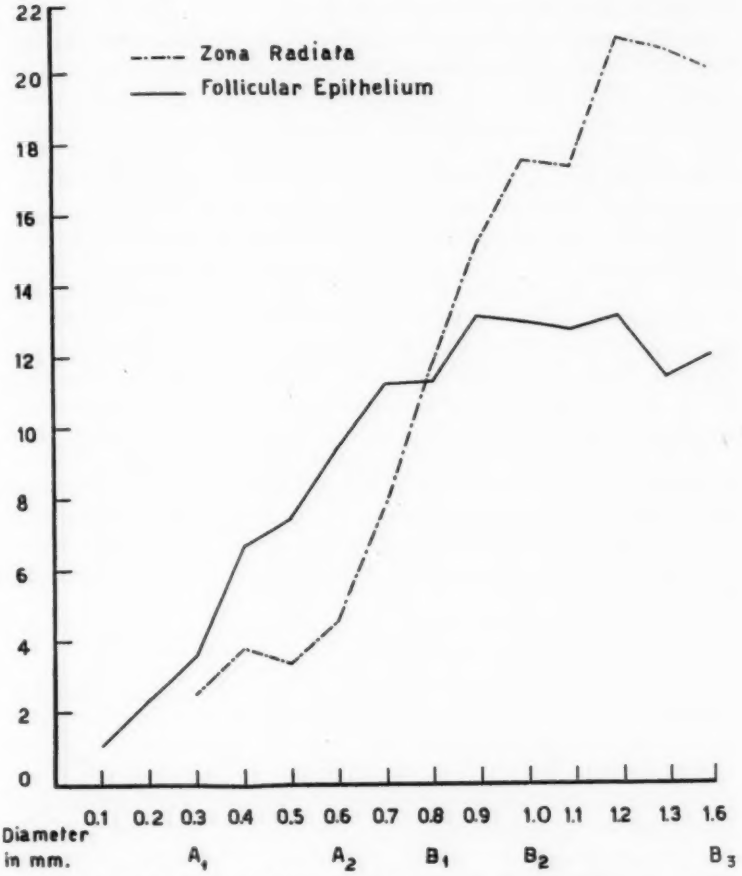


FIG. 19. Variation in height of follicular epithelium and zona radiata of the oöcyte during growth-period. The height in microns is indicated on the vertical axis.

was measured (Fig. 19). The smallest oöcytes, from 10 to 50 microns in diameter, do not have a continuous covering of follicle cells, although some squamous cells may be associated with them (Fig. 1).

When the follicle is complete, in oöcytes from 100 to 150 microns in diameter, its cells do not exceed 1.5 microns in height. During this period the cells of the follicle exercise no inhibitory effect upon the entrance of substances into the oöcyte. The quantity of potassium and acid proteins is relatively very great.

Maximum growth and differentiation of the follicle occurs during Period 2 A, and the height of the cells rises to 11 microns in 2 B_1. The changes in potassium and protein content have been noted. Obviously chemical changes are correlated with the differentiation of the follicle cells. During Period 2 A the large vesicles which are the earliest indication of the unique differentiation of the oöcytes appear, first at the periphery and then throughout the cytosome. One may assume that no longer are the constituents of cytoplasm being delivered in the same relative proportions. Certain substances are accumulating in excess of others. That this is conditioned by the composition of the follicle cells is a simple assumption suggested by the evidence at hand. When the deposition of yolk is occurring rapidly and directly in Period 2 B the follicle undergoes slight change. Some decrease in height is observed as the oöcyte reaches a maximum diameter, and nuclear regression is apparent. The amount of albumins is lowered during this stage.

In contrast to the follicular epithelium, the zona radiata continues to increase in thickness until the end of the growth-period. Some substances can be identified in its canals, but obviously nothing is stored in it. The observations made during this investigation do not suggest that the zona radiata functions in the selection of materials entering the oöcyte.

Summary

The plasmal and nucleal tests and the histochemical methods for detection of iron, potassium, and acid proteins indicate that changes in quality, quantity, and localization of substances characterize the period of differentiation in the oöcyte of *Fundulus*. The oöcyte at the end of its growth-period is morphologically very different from the oögonium. Its structural changes are found to be correlated with the metabolic activities leading to food storage. Cytosomal differentiations are conspicuously chemical phenomena in egg-cells.

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THE DEVELOPMENT OF THE PITUITARY GLAND IN FUNDULUS

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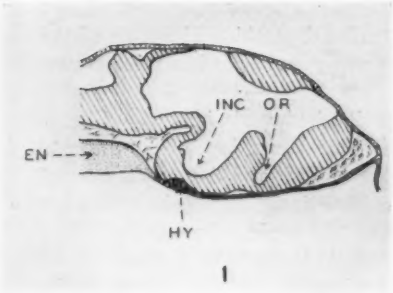
In discussing the structure of the pituitary gland of teleosts Stendell (1914) remarks that in no other group of vertebrates does the hypophysis present such variations, for in nearly every species within this group a different type of structure is encountered. While these can all be reduced to a single generalized plan it is not easy to determine accurately what structures are comparable in the glands of different species. This difficulty is obvious when the glands of *Esox* and of *Fundulus* are compared. According to Stendell, in the pars glandularis of the pituitary gland of *Esox* there is found a small "Hauptlappen" (a term frequently used as a synonym with "pars anterior"; see Tilney, 1936) which is restricted to the anterior dorsal part of the organ, a pars intermedia forming the caudal or ventro-caudal third of the entire gland, and between the two is a region found only in the teleosts and in *Petromyzon,* an "Uebergangsteil." In *Fundulus,* however, (Matthews, 1936) the pars glandularis presents only two distinct regions, a pars intermedia bordering processes of the pars nervosa and forming the posterior pole of the gland, and another part which comprises the major portion of the gland. Does this represent the pars anterior or the Uebergangsteil of other teleosts? A comparison of the cellular structure of this region with that of the pars anterior and the Uebergangsteil of *Esox* is of little value in homologizing these parts, for in *Esox* both the pars anterior and the Uebergangsteil are composed of basophilic cells in general with acidophiles restricted to the neighborhood adjacent to blood vessels, while in *Fundulus* the cells of this major part of the gland are predominantly acidophilic. In its relative size, however, this region in *Fundulus* compares more favorably with the Uebergangsteil, for it is certainly the largest part of the gland (Fig. 5) and Stendell states definitely (p. 92) that the Uebergangsteil is decidedly more voluminous than the principal lobe. Because of this, in describing the structure of the adult gland, I labelled this part the "transitional region," although the appropriateness of the term was questioned. It was thought that a study of the development of the hypophysis in this form might demon-

strate which of the parts found in the pituitary gland of other teleosts was lacking in *Fundulus*. Accordingly a series of embryos and young animals ranging in age from two to thirty-eight days after fertilization were fixed, sectioned and studied.

The rudiment of the glandular portion of the hypophysis can first be distinguished five days after fertilization as a small, flattened plate of ectodermal cells lying in close contact with the ventral surface of the forebrain in the region of the future infundibulum (Fig. 1). At this stage of development the foregut is represented by thick cellular masses in which cavities are beginning to appear. Unicellular gland cells described by Armstrong (1936) are already present. In animals six days old this flat plate of ectodermal cells has increased in size, and pushed upward away from the ectoderm so that it now lies slantwise against the posterior ventral angle of the infundibulum which is now quite definite. This epithelial rudiment of the hypophysis is still connected with the ectoderm by a well-defined stalk. These same relationships are present in animals seven days old.

Eight days after fertilization rather pronounced changes in the developing brain can be noted, chief among which is the first appearance of fibrous material in the ventral portion. The epithelial portion of the hypophysis is larger in size and more posterior in position, lying between the posterior wall of the infundibulum and the solid mass of cells which later becomes the foregut and is now heavily marked with the deep-staining unicellular gland cells mentioned above. At nine days of age the epithelial portion of the hypophysis is still further posterior in position (Fig. 2) and the stalk which connects it with the ectodermal region from which it first arose is somewhat longer. The small spaces which formed the first lumen of the gut now form a definite gut cavity but this does not communicate with the outside. At eleven days of age, when the foregut has extended up to the anterior end of the embryo and its lumen is closed off by only a thin membrane, the epithelial portion of the hypophysis lies still more posterior in position although maintaining its relationship to the infundibulum with which it is in close contact dorsally. It is in contact with the entoderm of the gut ventrally and a long, thin strand of ectoderm still connects it with the ectoderm at the anterior end of the embryo. In some cases at this age a few fibers may be found connecting this developing glandular portion of the hypophysis with the infundibulum, although in most cases these fibers appear in later development. Cell boundaries in the developing gland are still indistinct, as they have been throughout the course of development. Between the eleventh and twelfth days of age the strand of ectoderm which has previously connected the epithelial portion of the hypophysis with



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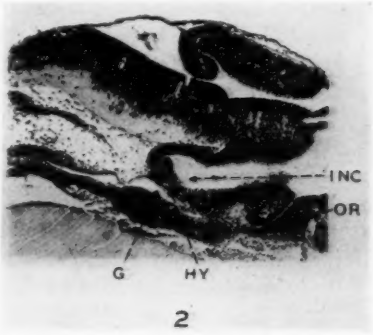

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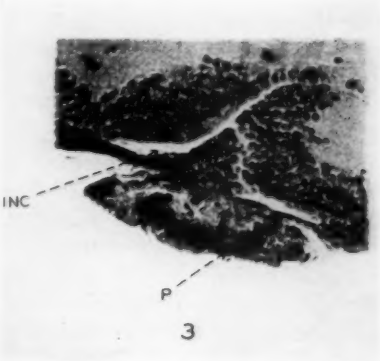

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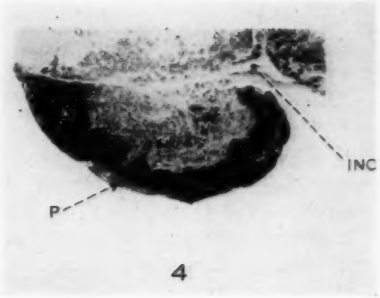

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FIG. 1. *Fundulus* embryo 8 days old, sagittal section of anterior end. Camera lucida outline, × 83. *EN*, entoderm; *HY*, hypophysis; *INC*, infundibular cavity; *OR*, optic recess.

FIG. 2. Embryo nine days old, sagittal section of anterior end, × 85. *HY*, hypophysis; *INC*, infundibular cavity; *OR*, optic recess; *G*, gut.

FIG. 3. Fundulus pituitary gland thirty-two days old, × 315. *INC*, infundibular cavity; *P*, pituitary gland.

FIG. 4. *Fundulus* pituitary gland approximately one year old, × 155. *INC*, infundibular cavity; *P*, pituitary gland.

FIG. 5. *Fundulus* pituitary gland, adult animal, 60 mm. long, sagittal sec, × 50. *INC*, infundibular cavity; *PA*, pars anterior; *PI*, pars intermedia; *PN*, pars nervosa.

the ectoderm at the anterior end of the embryo becomes interrupted and can no longer be traced in embryos twelve days old. The floor of the infundibulum dorsal to the developing pars glandularis has thinned considerably.

From this stage on changes in the developing pituitary gland occur more slowly. By the time the stomodeal membrane has broken through, which occurs in this series between the twelfth and thirteenth days of development, the epithelial part of the hypophysis has attained its adult position in relation to the pharynx and to the brain. It now lies on the ventral surface of the midbrain immediately beneath the ventral wall of the infundibulum, and fibers from the infundibulum join it to the brain. These fibers increase in number until, thirty-two days after fertilization, a short but fairly definite infundibular stalk can be distinguished. These fibers occupy the dorsal border and central portion of the hypophysis, forming the rudiment of the pars nervosa, with the cells that will produce the pars glandularis restricted to the periphery of the organ (Fig. 3). Blood vessels invade the gland between the fifteenth and sixteenth days after fertilization.

During this development of the pituitary gland in *Fundulus* no cavity is to be found at any time. The process in this animal thus differs from that described by Haller (1898), who observed a number of small spaces which appeared in the developing hypophysis of *Salmo,* these spaces eventually coalescing to produce an hypophysial cavity.

The cells of the developing glandular portion of the hypophysis are closely packed, with indistinct cell membranes. Although they do become larger as development proceeds, in the oldest of the animals reared in the laboratory, 38 days of age, the cells are still closely packed around the periphery of the organ and cell membranes are indistinct, with no secretory cells present comparable to those found in the pituitary gland of the adult. The smallest animals in which such cells have so far been found were collected in July, maintained in the laboratory for two months, and measured 26 millimeters when killed in September (Fig. 4). In the pituitary gland of these animals the cells of the pars glandularis are still located around the periphery but they are larger and have definite granules in their cytoplasm. The pars nervosa has also increased in size and processes extend from it into the pars glandularis. Since these animals were collected rather than raised in the laboratory their exact ages are unknown, but in all probability they had been spawned the previous summer and were approximately one year old. Obviously secretory cells first appear sometimes between the sixth week and the twelfth month of development. The exact time of their appearance and their coincidence with other features of development with which begin-

ning activity of the pituitary gland might be concerned must be determined in a series of animals hatched and maintained in the laboratory for longer periods.

Stendell (p. 109) emphasizes the fact that during the development of the pituitary gland of teleosts no cavity is formed. The cells of the solid ectodermal outgrowth proliferate rapidly at its anterior and posterior poles to form the pars anterior and the pars intermedia respectively. As a result of this polar proliferation of cells the region between the anterior and posterior poles, which is less active in development, presents characteristics transitional in nature between the pars anterior and pars intermedia, and constitutes the Uebergangsteil. But here in *Fundulus* apparently not only cells at the anterior and posterior poles but all the peripheral lying cells multiply to produce the pars glandularis. From a comparison of the pituitary gland of year-old animals (Fig. 4) with the developing gland in animals 32 days of age on the one hand (Fig. 3) and with that of adult animals on the other (60 mm. in length, Fig. 5) it is apparent that the main mass of the pars glandularis is produced by multiplication of the peripheral cells found in the early developmental stages. Later those cells adjacent to the pars nervosa may be distinguished from the others, particularly at the posterior pole of the gland where they form a large mass. This is undoubtedly the pars intermedia. The remainder of the pars glandularis is of uniform character. Neither in its development nor in its adult structure can it be called a transitional region between two other parts of the gland. Hence there exists no apparent reason for labelling this major part of the gland an Uebergangsteil. Because of this I now believe that the choice of the term "transitional region" for this major part of the gland of the adult is an unfortunate and confusing one. As a matter of fact, the anterior portion of the gland of *Esox* as described by Stendell differs so much from that part of the gland in *Fundulus* that on the basis of structure alone it is uncertain what parts are comparable in this region of the two glands. Since in *Fundulus* this part in question forms the anterior portion of the organ it may appropriately be called the pars anterior, although it is doubtful whether it is homologous with Stendell's "Hauptlappen."

Summary

The epithelial portion of the pituitary gland of *Fundulus* arises from ectodermal cells that proliferate to form a solid mass which comes to lie between the infundibulum and the pharynx. No lumen occurs in this mass of cells. After fibers from the infundibulum have entered this mass the cells are restricted to the periphery where they multiply to

produce the pars glandularis of the adult gland. Those cells adjacent to the pars nervosa can be distinguished from the rest of the glandular portion and constitute the pars intermedia. The remainder of the pars glandularis is of uniform character. No "transitional region" can be distinguished in it either in the developing or in the adult gland. Since this portion forms the anterior part of the organ it may appropriately be called the pars anterior.

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THE SUPPRESSION OF CLEAVAGE IN ASCARIS EGGS BY ULTRACENTRIFUGING[1]

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It has long been known that the division of the cytoplasm of the living egg can be stopped by many external agencies (cold, lipoid solvents, hypertonic and hypotonic solutions, mechanical agitation, etc.) without necessarily inhibiting the division of the nucleus (Wilson, 1902; Chambers, 1924 for references). Furthermore, it is also true that cytoplasmic cleavage may take place in the absence of nuclear material (Harvey, 1936). Accordingly, it is evident that, although nuclear and cytoplasmic divisions are usually correlated they may, nevertheless, be quite independent processes, neither of which is fundamentally dependent upon the other.

In some recent experiments (King and Beams, 1937) on the effects of strong centrifugal force upon the early cleavage stages of *Ascaris* eggs, particularly the diminution process, it was noted that many eggs undergo typical nuclear division in the ultracentrifuge without the accompanying division of the cytoplasm. We are reporting here these experiments together with an analysis of the results in the event that they may have some bearing upon the problems involved in the mechanics of mitosis.

Material and Methods

The eggs used in these experiments were taken from the anterior one-half inch of the uteri of *Ascaris equorum* (=*megalocephala*) variety *univalens*,[2] which had been kept in a refrigerator at 5° C. for not longer than thirty days. Microscopic examination of the eggs taken from this portion of uteri showed them to be in the pronuclear stage of development.

Some of the eggs from each batch were placed in the rotor of the air-driven ultracentrifuge (Beams, Weed, and Pickels, 1933) while in the pronuclear stage, and were centrifuged in 0.9 per cent salt solution at

[1] Aided by grant from the Rockefeller Foundation for work on Cellular Biology.

[2] It is indeed a pleasure to express here our indebtedness to the authorities of the Hill Packing Company at Topeka, Kansas, for kindly supplying us with the worms.

approximately 150,000 times gravity for varying lengths of time, the maximum being 10 days. In addition, observations were made upon the eggs while centrifuging by means of the centrifuge-microscope recently described by Pickels (1936). In other experiments the eggs were placed under the same conditions in an electric-driven centrifuge, which developed a force of approximately 5,000 times gravity. Part of the eggs from each batch used in an experiment were always kept as controls. All the experiments were done at room temperature which varied from 20° to 25° C.

Sealed cover-glass preparations of the normal and experimental living eggs were studied under the oil immersion lens. It was found that centrifuged eggs placed in 10 per cent formalin solution at 55° C. were coagulated so that the various conditions of stratification are preserved and may be studied at leisure. When permanent slide preparations were made, the eggs were killed in a mixture of 4 parts of 95 per cent alcohol and 1 part of glacial acetic acid and subsequently stained in Heidenhain's hæmatoxylin. In an effort to analyze the components of the various stratified layers a few eggs were killed in Champy's solution at 55° C., and afterwards impregnated in 2 per cent osmic acid for 5 days. By use of antiformin it is possible to remove the shell from living eggs without apparent injury to them.

Description

After sperm entrance and the completion of maturation in *Ascaris*, the eggs shrink and round up giving rise to a rather large perivitelline space which is filled by a watery fluid and enclosed by a very resistant shell (Plate I, Fig. 1). The oöplasm of the egg at this time is heterogeneous in structure, being composed of small brown granules, granular and short rod-like mitochondria (?), clear spherical vacuoles, oil globules and an optically homogeneous cytoplasm. The pronuclei are transparent, rounded bodies located in or near the center of the egg. Surrounding the egg is a delicate plasma membrane, the morphology of which is not discernible even under the oil immersion lens.

Upon centrifuging at 150,000 times gravity the eggs are stratified almost immediately into the following layers: (1) oil globules at the centripetal pole, (2) a zone of clear vacuoles, (3) transparent cytoplasm, and (4) brown granules at the centrifugal pole (Plate I, Fig. 2). The nuclei and also the mitotic spindle in dividing eggs are included in the clear cytoplasmic layer. The mitotic spindle is oriented at right angles to the centrifuge axis and parallel to the stratified layers. Unlike the condition in embryonic chick cells, the elements of the mitotic spindle

are not distorted. This indicates that their relative specific gravity is approximately the same as that of the cytoplasmic layer. When viewed through the microscope-centrifuge, the eggs were observed to be somewhat elongated along the centrifuge axis, so that the centrifugal and centripetal ends of the eggs are in direct contact with the shell, which no doubt aids in preventing their fragmentation. Eggs in the pronuclear stage may, in rare cases, be divided into three parts within the shell as follows: fat at the centripetal pole, protoplasm and clear vacuoles in the middle and brown granules at the centrifugal pole (Plate I, Fig. 3). In other more frequent cases only the oil globules are completely rounded off from the rest of the egg at the centripetal pole, and flattened in the form of a disc (Plate I, Figs. 6, 7, 8, 9, 11). Eggs of this kind, if removed from the centrifuge before cleaving, frequently develop into normal larvæ, with the flattened disc of oil globules within the perivitelline space. However, if the eggs are removed from the centrifuge before the oil globules are completely cut off they become reincorporated into the egg through a narrow stalk, and eventually are redistributed as are the other layers of the egg. In Plate I, Fig. 4, is illustrated a centrifuged egg with conjugating pronuclei that had been killed in Champy's solution and subsequently impregnated in 2 per cent osmic acid. It will be noted that a dark layer, which is thought to contain the mitochondria, appears just centripetal to the brown granules. This layer of small granules can also be seen in the living egg under suitable conditions. It had previously been thought that the brown granules represent the mitochondria in *Ascaris* eggs (Fauré-Fremiet, 1913). The dark granular layer just centrifugal to the fat globules may possibly represent the Golgi material.

If the eggs are left in the centrifuge until the controls have undergone cleavage a typical mitotic apparatus (centrosomes, asters, spindle and chromosomes) is formed (Plate I, Fig. 5), giving rise to two nuclei within the cell because of the failure of the cytoplasm to cleave. (The cytology of the mitotic figure and the chromosome behavior in such eggs is to be reported elsewhere—King and Beams, 1938.) These two nuclei, in turn, may form typical division figures simultaneously (Plate I, Fig. 6), giving rise to four nuclei within the uncleaved egg (Plate I, Fig. 8); the four nuclei may divide in the same manner (Plate I, Fig. 11), each division corresponding to a cleavage in the controls. In fact, the first two nuclear divisions often take place at about the same rate as the first two cleavages of the controls. However, the subsequent cleavages often become more or less irregular and out of step with the controls.

If the eggs are removed from the centrifuge after one, two or more divisions of the nuclear material only, a curious surface phenomenon is

noted in the region of the clear cytoplasmic layer. Immediate examination of the eggs upon removal from the centrifuge often reveals many small and a few quite large pseudopodium-like processes flowing out of the egg; these are in many cases completely cut off (Plate I, Fig. 7). This material which has been stratified is thought to be or to give rise to the "surface active material" which breaks out and precipitates, forming a film or plasma membrane. The presence of such a material in the egg is assumed to be necessary in the regions where the cleavage fur-

PLATE I

Explanation of Figures

All the figures were drawn from living eggs by Mary Crosten. The amount of the centrifugal force has been in all cases 150,000 times gravity unless otherwise indicated. The direction of the centrifugal force in all cases except Fig. 1 was approximately toward the bottom of the plate.

Fig. 1. Pronuclear stage of a normal *Ascaris* egg showing the general structure of the cytoplasm and the position of the pronuclei.

Fig. 2. Egg as in Fig. 1 after centrifuging for one-half hour. Materials stratified with fat at the centripetal pole, then a layer of clear vacuoles, next a layer of clear cytoplasm containing the pronuclei, and finally, a layer of brown granules at the centrifugal pole.

Fig. 3. Unusual condition of egg fragmented into three parts: fat and clear vacuoles in the centripetal fragment, cytoplasm and pronuclei in the middle fragment and the brown granules in the centrifugal fragment. Centrifuged two hours.

Fig. 4. Egg fixed in Champy's solution immediately upon removal from the centrifuge, and subsequently impregnated in 2 per cent osmic acid. Stratification clearly shown. The dark layer between the nucleus and the brown granules is thought to represent the mitochondria. The darkly impregnated layer just centrifugal to the fat globules may possibly represent the Golgi material.

Fig. 5. Egg undergoing division of the nucleus without division of the cytoplasm. The asters are faintly shown.

Fig. 6. Prophase stage of the two nuclei which correspond to those in the second cleavage of the controls. Small masses of "surface active material" shown budded off from the egg.

Fig. 7. Drawing of egg removed from centrifuge after the controls had undergone one division. The formation of the pseudopodia in the region of the cytoplasmic layer is shown.

Figs. 8–9. Eggs that have undergone two mitotic divisions of the nuclear material without division of the cytoplasm in the centrifuge. Large fragments are shown which were actively undergoing ameboid movement. The oil globules are shown fragmented from the egg at the centripetal pole. Drawings made after a partial redistribution of the stratified layers had taken place.

Fig. 10. Egg removed from centrifuge at the time the controls had undergone the first cleavage. Many pseudopodium-like processes shown at the periphery of the egg, none of which in this particular case was fragmented off. Drawing made after partial redistribution of the stratified layers.

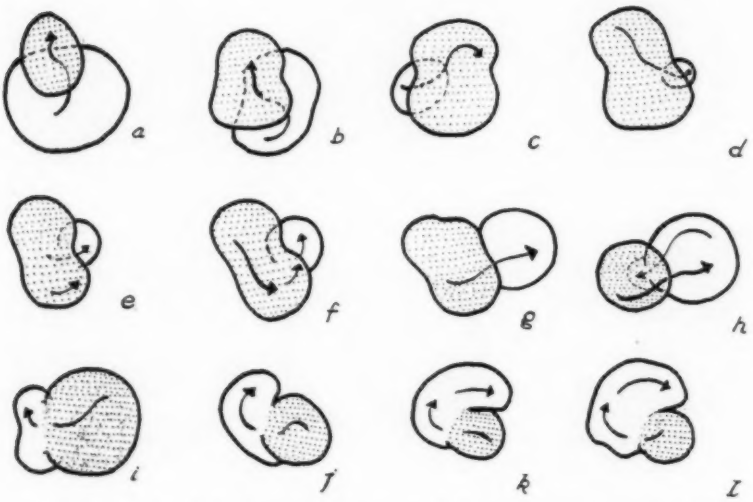
Fig. 11. Egg that had passed through three mitotic divisions of the nuclear material in the centrifuge. One nucleus is seen cleaved off into a small cell of clear cytoplasm. A redistribution of the brown granules in the egg has taken place. Fat globules are shown at the centripetal pole.

Fig. 12. "Ball" egg that divided in centrifuge at 5,000 times gravity. Most of the brown granules are cleaved off during the first division. The stratification of the various materials is clearly shown.

PLATE I

rows are destined to form. The fragments show very striking and rapid ameboid movement within the perivitelline space. In text fig. 1 (*a–l*) are illustrated diagrammatically the consecutive movements of a typical fragment like that shown in Fig. 8 during an interval of 5 minutes. Such active movement has been noted to continue for four or five hours, after which time the fragments may divide into many smaller ones. We have not observed that these bodies subsequently fuse with the egg.

In other less frequent cases, where the eggs were treated in a similar way, many small pseudopodium-like processes are formed at the surface



TEXT FIG. 1, *a–l*, diagrams showing the ameboid movement of a typical fragment like that shown in Plate I, Fig. 8, during an interval of 5 minutes.

of the clear and granular layers but they do not become separated off (Plate I, Fig. 10). Such pseudopodia upon division of the egg outside of the centrifuge are reincorporated with the blastomeres. It is an interesting fact that frequently one observes eggs (such as those shown in Plate I, Fig. 11), where one or more small cells with clear cytoplasm have been cleaved off in the centrifuge. This condition probably occurs when one of the mitotic spindles is sufficiently close to the periphery of the egg in the presence of "surface active material."

Those eggs that have undergone one or two divisions of the nuclei without division of the cytoplasm in the centrifuge will, upon removal

from the centrifuge, frequently divide into twice as many cells as there were nuclei present. This division, however, takes place only after partial or complete redistribution of the stratified materials.

Ascaris eggs like those of the sea-urchin (Harvey, 1933) will divide in a gravitational field of 5,000 times gravity. In most of the eggs the cleavage is complete. However, in a few it is incomplete, as it does not pass through, but around the brown granular layer at the centrifugal pole, giving rise to what Boveri (1910) and Hogue (1910) have called "ball" eggs (Plate I, Fig. 12). The fact that these eggs undergo cytoplasmic cleavage may be explained by assuming that the force used, although sufficient to produce stratification of the visible materials, has not been great enough to displace and stratify the "surface active material." This assumption is supported by the fact that eggs centrifuged at 5,000 times gravity while dividing do not usually form pseudopodia as do those centrifuged at higher speeds.

No attempt has been made here to determine the absolute viscosity of the protoplasm of *Ascaris* eggs. However, the relative viscosity has been determined at room temperature by the centrifuge method, and by observations upon Brownian movement in the eggs at various stages in the mitotic cycle. Fauré-Fremiet (1913) has also used the centrifuge method to study the viscosity of *Ascaris* eggs at different temperatures. In our experiments the eggs, which were in different stages of division, were placed in the electric machine and centrifuged at 5,000 times gravity for 20 minutes. The relative position or layering out of the brown granules was used as an index of the relative viscosity of the eggs, in the pronuclear stage, prophase, metaphase, anaphase and telophase. Brownian movement was followed in eggs that were undergoing division, as was the redistribution rate of the brown granules after they had been displaced by the centrifuge.

The evidence from these observations indicates that the egg is relatively fluid during the pronuclear and prophase stages. During metaphase the egg becomes progressively more rigid and Brownian movement usually ceases except perhaps at the periphery. During anaphase and telophase the eggs show the highest degree of viscosity. At the end of telophase when the asters begin to recede, and the reorganization of the nuclei takes place, the viscosity gradually becomes low again.

It should be pointed out that, during the prophase, when the amphiaster is forming, a gelation or relatively high viscosity is present in the region of the immature or small aster. It seems probable that the viscosity is as high in this region as it will ever be, and that the following stages, which show a higher viscosity for the egg as a whole, are due primarily to a spreading of this gelation from the region of the small

aster toward the periphery of the egg, a condition associated with the development and growth of the asters. (For a recent review of the literature on viscosity studies see Fry and Parks, 1934.)

Discussion

Cell division under normal conditions appears to be accomplished by a series of complex integrated phenomena, involving both the nucleus and cytoplasm; this becomes manifest, in part at least, through changes in viscosity, appearance of the mitotic figure, and cleavage furrow. These morphological changes in the dividing egg are, no doubt, the expression of what might be termed a "mitotic field of force," by which a part of the work necessary for cell division is accomplished. It is, however, beyond the scope of this paper to discuss the whole mechanism of cell division, but a few remarks upon the process of cytokinesis in the light of our results seem desirable.

It should be pointed out at the beginning that the failure of the *Ascaris* eggs to cleave in the ultracentrifuge can not be attributed to any marked change in the morphology or position of the mitotic figure, as its form is exactly comparable to that of the normal egg, and its position in the cytoplasmic layer is the same as that in eggs which undergo cleavage in the centrifuge at 5,000 times gravity. Furthermore, contrary to the views expressed by some workers (Chambers, 1924; Harris, 1935), no evidence was observed which would indicate that the strong centrifugal force used in our experiments did in any way affect the normal viscosity cycle during nuclear division. In this respect our results differ from those of most other investigators who have inhibited cell division by external agencies (cold, ether, hypertonic and hypotonic solutions, mechanical agitation, etc.), in that these methods affect the viscosity of the cell, resulting in a partial or complete disintegration of the asters, which, in turn, inhibits cell division. In other words, "cells which possess asters under normal conditions absolutely depend upon the presence of asters for cytoplasmic division" (Chambers, 1924). It is evident, therefore, that the inhibition of cytoplasmic cleavage in the *Ascaris* egg by the ultracentrifuge can not be explained upon the same basis as the cases cited above.

It is a well established fact (Erlanger, 1897; Conklin, 1917; Speck, 1918; Strangeways, 1922; Chambers, 1924; Gray, 1931; Harris, 1935, and others) that in many dividing cells, particularly the nematode egg, there are important peripheral changes which take place in the cytoplasm, which seem to be associated with the development of the amphiaster, and which have a distinct bearing upon cell division (see Speck,

1918 and Chambers, 1924 for literature). In general, it may be said from the results of the above-named investigators, that when the asters develop to the point of almost reaching the periphery of the egg, a decrease in peripheral viscosity becomes noticeable. In this relatively fluid periphery it is possible to see a flow, initiated by a change in surface tension, which, according to all the investigators who have described it, is directed toward the equatorial region of the cell. It is not possible in the *Ascaris* egg to observe a flowing movement in the peripheral region during division, but it is significant that here, particularly at the equator, cessation of Brownian movement is generally last observed during metaphase. Furthermore, in a few instances, it was found that, during the normal mitosis of *Ascaris* eggs, a distinct " bubbling " or protrusion of a relatively clear material in the form of small pseudopodia was observed, especially at the equatorial region. Kautzsch (1912), and Painter (1915) have likewise described marked conditions of this kind in *Ascaris* eggs in which, apparently, the normal division had been delayed. This activity or mobility at the surface of dividing cells has frequently been observed, particularly in tissue culture cells (Strangeways, 1922).

It seems not unreasonable to suppose, therefore, that the relatively fluid peripheral material is formed during the gelation of the amphiaster, or at the solution of the nuclear membrane, and then is forced to take up a position at the periphery of the egg, where it seems to function in the formation of the cleavage furrow.

The fact that the marked formation of extraovates takes place in the region of the cytoplasmic layer of eggs, that have undergone one or more typical nuclear divisions in the centrifuge although cleavage has been suppressed, may be explained by the assumption that the peripheral surface active, membrane-forming material is stratified in this region. Our observations indicate that at each division of the nucleus more of the " surface active material " is formed, and, if in the centrifuge, is stratified so that when the centrifugal force is released, some of this material, which changes surface tension, is extruded, and frequently is completely separated from the egg. Ameboid activity ensues until all the surface energy is dissipated. If this interpretation is correct, and, if the surface active material is essential for film or membrane formation, as there is good evidence for believing (Heilbrunn, 1928), then one need only assume that the inhibition of cleavage of the *Ascaris* eggs in our experiments is due to a concentration of the material into a layer parallel to the axis of the mitotic spindle, thus depleting the egg of the membrane-forming material in the regions where the cleavage furrows would normally form. This interpretation, we believe, is in harmony

with the highly significant work of Heilbrunn (1928) on the "surface active material" of *Arbacia* eggs. He found, after stratifying of the visible materials in the eggs by means of the centrifuge, and subsequent crushing, that a surface film was formed only at the centrifugal pole, the position taken up by the pigment granules. Heilbrunn, therefore, concluded that the "surface active material" had been displaced in the centrifuge, and that it was the pigment granules or material stratified in the same region which is or gives rise to the "surface active material."

There are, no doubt, some who will interpret the failure of the cytoplasm to cleave in the centrifuge as simply mechanical, because of the intense stratification or "packing" of the elements in the stratified layers. This condition of stratification may possibly be a factor; but it has been shown by Boveri (1910), Hogue (1910), and in this paper, that cleavage will take place in the centrifuge when the visible materials are clearly stratified. It is only when the centrifugal force has been very great that division fails to occur. Why the cytoplasm fails to cleave in the region of the dividing mitotic figure is not known, but one might assume that the "surface active material" is stratified into a layer which does not include the mitotic spindle; or perhaps because of its stratification, the "surface active material" has become temporarily inactivated.

It should be pointed out that in certain biological material, the formation of a plasmodium or syncytium is the normal condition. For example, in the developing insect egg, the first divisions involve the nucleus only; the cleavage of the cytoplasm takes place later after the nuclei have migrated to the surface of the egg. No satisfactory explanation for this condition has ever been suggested, but might it not be because of the lack of a sufficient quantity of some material essential for cytoplasmic cleavage, such as, for instance, the surface active, membrane-forming material? Or could it be that the materials essential for cytoplasmic cleavage can not function properly in the interior of the egg?

In a preliminary report (Beams and King, 1936), it was shown that eggs of *Ascaris suum* were not killed by centrifuging at 400,000 times gravity for 30 minutes; the same condition holds true for eggs of *Ascaris equorum*. These facts are interesting in the light of the recent work of Svedberg (1934) on strongly centrifuging non-living colloidal systems. He found that certain of the large molecules may be thrown out of solution at 75,000 to 200,000 times gravity. Tobacco virus proteins may be likewise thrown out of solution and crystallized at 200,000 times gravity (Stanley and Wyckoff, 1937), and recently studies have indicated that it might be possible to separate isotopes by the ultracen-

trifuge method (Beams and Haynes, 1936). One might assume, therefore, from these facts, that a similar separation of the light and heavy molecules might take place in protoplasm at such strong forces. In fact, Moore (1935) has recently concluded that the living elements of a plasmodium consist of heavy and light components, which, when centrifuged at 75,000 times gravity for 5 minutes, are separated, and proliferation stopped. However, if allowed to rest for sufficient time, the separated elements return to their normal spatial relationship and proliferation is resumed. Our work shows definitely that *Ascaris* eggs will live and undergo nuclear division in the centrifuge at 150,000 times gravity for at least ten days. Because of this fact we do not believe that any marked separation of protoplasmic structure in the manner suggested by Moore has taken place. On the other hand, it seems more likely that forces, perhaps electro-static in nature, are sufficiently great in *Ascaris* protoplasm to prevent a disruption of its architecture by such strong centrifugal forces, or, less likely, perhaps the living units of the protoplasm may all be of the same specific gravity.

Conclusions

1. Eggs centrifuged at 5,000 times gravity are stratified into the following layers: (1) fat at the centripetal pole; (2) clear vacuoles; (3) clear cytoplasm containing the pronuclei or the mitotic spindle; the latter usually oriented with its long axis at right angles to the centrifugal force and parallel to the layers; and (4) brown granules at the centrifugal pole. When centrifuged at 150,000 or 400,000 times gravity and subsequently fixed in osmic acid solution, two additional layers are noted: one just centripetal to the brown granules, the other just centrifugal to the fat layer; the former is believed to represent the mitochondria; the other may possibly represent the Golgi material. At these high speeds the brown granules rarely become fragmented from the egg. However, the oil globules are frequently cut off at the centripetal pole.

2. Eggs in the pronuclear or early prophase stages will, upon being placed at 5,000 times gravity, undergo the early cleavages at about the same rate as controls. In a low percentage of eggs the brown granules are cut off at the first cleavage, which usually takes place at right angles to the stratified layers.

3. Eggs in the pronuclear or early prophase stages that are centrifuged at 150,000 times gravity undergo typical mitotic division of the nucleus without the usual division of the cytoplasm. This gives rise to two nuclei within the egg, which may in turn form typical mitotic

figures and divide at the same time, giving rise to four nuclei within the uncleaved egg. The first two or three divisions of the nuclear material often correspond in tempo to the first two or three cleavages of the controls.

4. Eggs that have undergone one or more divisions of the nucleus without the division of the cytoplasm show, upon immediate examination after removal from the centrifuge, a curious peripheral "bubbling," or the extrusion of pseudopodium-like processes in the region of the clear cytoplasmic layer. These pseudopodia may be cut off from the egg, in which case they undergo very rapid ameboid movement. If not detached they are withdrawn into the egg during the redistribution of the stratified materials and subsequent cleavage.

5. The material, which is extruded in the form of pseudopodia, is thought to represent "surface active material"; a material which upon coming in contact with the surface or outside environment of the egg coagulates, giving rise to a film or plasma membrane. It is this same material which during normal cleavage concentrates at the periphery of the egg and functions in the formation of the cleavage furrow.

It seems probable that the increase or growth of the "surface active material" is not markedly inhibited by high speed centrifuging, so that at each division of the nucleus more of it is formed, and becomes stratified. Thus, upon cessation of the centrifuging, part of this material is extruded, a membrane is formed upon coming in contact with a foreign environment and active ameboid movement is initiated.

6. Uncleaved eggs centrifuged at 400,000 times gravity for 30 minutes are not killed, and, if the stratified materials are redistributed before cleavage takes place, they apparently develop normally.

7. No evidence was found which indicates a separation of the ultramicroscopic cytoplasmic components had taken place.

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A STUDY OF THE BACTERIAL AND ALLEGED MITOCHONDRIAL CONTENT OF THE CELLS OF THE CLOVER NODULE

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INTRODUCTION

In a paper recently published the author expressed the view that certain granular and rod-like cytoplasmic inclusions occurring in *Amœba proteus* and *Arcella vulgaris* belonged in the category of bacteria, probably symbiotic or commensal, while still other similar inclusions appeared to be only temporary bacterial invaders of the cytoplasm. Certain of these granular and rod-like inclusions had, in the past, been described as mitochondria. In the course of this investigation the initial intention was to employ the clover nodule as material for making a comparative study between certain cell inclusions, alleged mitochondria, as they appeared in the nodule, and similar inclusions occurring in *Amœba* and *Arcella*. From available literature the clover nodule promised to be very valuable material for making such a comparative study. Certain applied tests, however, induced me to discard that material for the time, with the hope of being able later to publish my findings separately.

The phenomenon involving bacteria and alleged mitochondria as it occurs in the nodule of the clover is quite puzzling. The problem of making a clear distinction between the two by present-day methods of technique has previously been recognized by investigators, including Buchner (1921).

Techniques, similar to that employed in arriving at the above-mentioned conclusions regarding *Amœba* and *Arcella*, have likewise been employed here in an intensive study of the contents of the clover nodule. Since this material can easily be secured throughout the year and since the nodules contain bacterial forms, including both rods and granules, varying in size from minute forms which approach the limit of microscopic vision to forms of relatively large size, it becomes quite evident that such material offers an excellent opportunity for the study of symbiotic relationship, as well as for making a comparative study of different cell constituents.

Mitochondria have been described in the nodule of clover in association with the different pleomorphic forms of recognized bacteria. Cowdry (1923) has presented characteristics which he considers serve to distinguish between small bacterial forms and mitochondria, as they occur in the nodule of the white clover. Differentiation between bacteria and alleged mitochondria has been made largely on the grounds of staining reactions and responses to well-known lipoid solvents. It is the purpose of this investigation to show that size, staining ability, reaction to Janus green, and lipoid solvents are not sufficient to distinguish bacterial forms and apparent mitochondria as they occur in the clover nodule.

From the following observations upon many nodules, treated with different methods of technique, smears, cultures on artificial media of bacteria from both the nodule and from the soil, I am convinced that an important bacterial type, or possibly types, as well as their general staining qualities, have been overlooked.

Wallin (1922) is of the opinion that a minute type of bacteria, not unlike mitochondria in appearance, exists in the nodule of the white clover. He designates them " juvenile forms " and believes them to be young bacilli which have recently entered the nodule from the soil. He asks (p. 458) : " What evidence may be submitted to indicate that these bodies, generally considered cytoplasmic organs, are not bacteria that have gained entrance to the plant through the root-hairs? The author has not been able to find any evidence that would satisfactorily answer these questions."

In the present paper, it is my aim to deal chiefly with the minute rod-like and granular inclusions occurring in the clover nodule and in the soil and to submit evidence which further indicates that minute forms of bacteria have been confused with mitochondria.

Material and Methods

Clover nodules were secured from various locations, during different seasons of the year, and from soils varying in moisture content. While the nodule of the white clover was chiefly used during this investigation, nodules of crimson clover were likewise studied. Mitochondrial fixatives, including Altmann's, Regaud's, Champy-Kull's, Flemming's, and Murray's, were employed. In addition to mitochondrial fixatives, Schaudinn's fluid with iron-hematoxylin was used. In some cases, prior to fixation, nodules were treated with 95 per cent alcohol or ether for 12 to 24 hours. Still others were fixed in Altmann's fluid to which had been added 5 per cent of acetic acid. In order to avoid as much as pos-

sible variations which naturally occur in structure and content of different nodules, as well as in different regions of the same nodule, nodules were severed longitudinally in nearly equal halves and the halves subjected to different treatment. The two halves of the same nodule were then placed side by side, embedded, sectioned, stained and mounted.

Nodules were always thoroughly washed in sterile water before cutting. After cutting on a slide, in a drop of sterile water, smears were made of that portion of the contents normally lost during the cutting procedure. The smears, likewise, served in making comparative studies. In addition to treating the nodule as a whole, smears of entire nodules were made. All smear preparations were allowed to dry completely before further treatment. While some were treated with alcohol or ether before applying mitochondrial fixatives, others were not.

Supravital tests were made on the contents of the clover nodule by thoroughly crushing the nodule upon a slide which had previously been filmed with a 1–20 alcoholic solution of Janus green (Grübler's) prepared from a 1 per cent stock solution in absolute alcohol. Comparative tests of the nodular contents were made by examining similarly treated nodules which were not exposed to Janus green. For permanent staining, anilin-fuchsin with methyl green or toluidin blue counterstain and iron-hematoxylin were employed.

In the following illustrations granules and rods which color red in the stained material are drawn in black, while those staining blue, green, or indefinitely are illustrated in diluted ink.

Artificial media were inoculated with contents of the nodule, as well as with soil which had been removed from the roots. Two different kinds of media were used for this purpose: a mannite medium, free from combined nitrogen and containing a fermentable carbohydrate, the formula for which will be found in Topley and Wilson (1932), p. 315, Vol. 1; for plating a 2 per cent agar plus 1 per cent lithium chloride.

I wish to make grateful acknowledgment to Dr. H. E. Jordan of the Department of Histology and Embryology, who placed at my disposal the facilities of his laboratory, for his very valuable aid and kindly criticism during the course of this investigation and preparation of this paper.

OBSERVATIONS

Wallin describes and illustrates three distinct types of bacteria-like organisms as they occur in the nodule of the white clover. One of these types, consisting of minute rods and granules, he found located in the distal or younger portion of the nodule. He states: "In Fig. 3 the cells contain small bodies that are not unlike mitochondria in appearance.

These forms, undoubtedly, are the young bacilli that have recently entered the nodule, or they represent a young form that will later metamorphose into the mature type. They may be designated the 'juvenile forms.'" Cowdry, on the other hand, thinks: "that Wallin's questions may be answered through the simultaneous and differential demonstration of mitochondria and Bacillus radicicola side by side within the same cells." In other words, Cowdry is of the opinion that mitochondria and bacteria can be distinguished in the nodule on the grounds of morphology, differential staining, arrangement in the cell, fixing with Bouin's fluid and 95 per cent alcohol. Cowdry also states: "In figures 1 and 2 only mitochondria are shown. They occur in about the same number in neighboring cells and are, for the most part, rod-like, and occasionally filamentous, not spherical, as indicated by Wallin in his figures 6 and 7."

Among the many nodules examined during the present investigation, much variation in nodular contents has been encountered. In matter of fact so much variation occurs with respect to cellular structure, nature, and arrangement of cellular contents, morphological bacterial types, rods and granules, differential staining, that one is at a loss to determine just what constitutes the rule. Nodules occur which have little cellular arrangement, the cytoplasm being in a continuous, unlimited mass, in so far as the cell walls are concerned. Minute rods and granules sometimes occur in this cytoplasmic mass in abundance. In some sections the large majority stain red and have the appearance of mitochondria. In later sections of the same nodule apparently the same inclusions sometimes show more differential staining qualities, a lesser number staining red while more are stained green, until finally very few, if any, present a mitochondrial appearance, but stain green. Other nodules present relatively large cavities devoid of cytoplasm, or cells with little or no cytoplasm, and these may be partially or completely filled with bacteria-like inclusions. These inclusions may vary slightly in size in some cases, but many are very minute (Plate I, Figs. 1 and 2). They frequently stain red, while in some cases they show differential staining properties.

Minute rods and granules which color red occur in some cells which possess large metamorphic forms of *Bacillus radicicola* and are distributed peripherally, as Cowdry has shown, or they may be scattered more or less uniformly throughout the cell (Plate I, Figs. 3 and 4). Among these, one finds a certain amount of differential staining in some cases. I have been unable to find any evidence that strict peripheral arrangement of these minute inclusions in such cells is the rule, as Cowdry has emphasized. They appear as rods, short filaments, or granules.

Seldom does one find two nodules, even from the same plant-root, presenting the same picture with regard to bacterial or minute bacteria-like content. Different metamorphic forms of *B. radicicola* appear in the same nodule, but from the fact that I have never observed the entire metamorphic series within a single nodule, I conclude that a complete series within a single nodule is most probably an exception and not the rule. In fact, in many nodules, one single form seems to predominate throughout. Usually, there are several forms present.

Rods and granules, many of which are minute and approach the limit of clear microscopic vision, are found within some of the nodules. In some cells they are abundant, while in others they are sparsely scattered (Plate I, Figs. 5 and 6). In some nodules, apparently, they are absent, while in others only a small percentage of the cells possess them. Their staining ability varies, even within the same cell; i.e., some will stain red, while others stain green or blue (Plate II, Figs. 7 and 8).

The fact is well recognized that properties such as the above, alone, are not sufficient to determine the true nature of small cytoplasmic inclusions, making it necessary to seek additional evidence. One of the recognized criteria for demonstrating mitochondria after mitochondrial fixation is their ability to color red with anilin-fuchsin, followed by a counter-stain of methyl green or toluidin blue. A significant fact here is that, among the numerous nodules investigated during the present

EXPLANATION OF PLATES

All drawings were made with the aid of a Bausch and Lomb 1.8 mm. oil-immersion objective and a 10 × ocular. All figures with the exception of Fig. 18 (Plate III) were taken from preparations treated with mitochondrial fixatives and stained with anilin-fuchsin and counterstained with methyl green or toluidin blue. Figure 18 was taken from a preparation treated with Schaudinn's fluid and stained in iron-hematoxylin. Magnification about 1,200.

PLATE I

Explanations of Figures

FIG. 1. Section of a nodule with the lumen partially filled with bacteria-like rods which show some differential staining. Altmann's fixation.

FIG. 2. Section of a nodule with cells and lumen containing both rods and granules. Shows some differential staining. Regaud's fixation.

FIG. 3. Cells from a nodule, showing mature forms of *B. radicicola* together with numerous minute granules and rods located in different regions of the cell. Shows some differential staining. Regaud's fixation.

FIG. 4. Two cells from a nodule, showing minute granules and rods scattered among the mature bacterial forms. Murray's fixation.

FIG. 5. Cell from a nodule, showing minute cell inclusions. Flemming's fixation.

FIG. 6. Section of a nodule, the cells of which contain many rods and granules. The greater number stain red, a few green, while some are indefinitely stained. Champy-Kull's fixation.

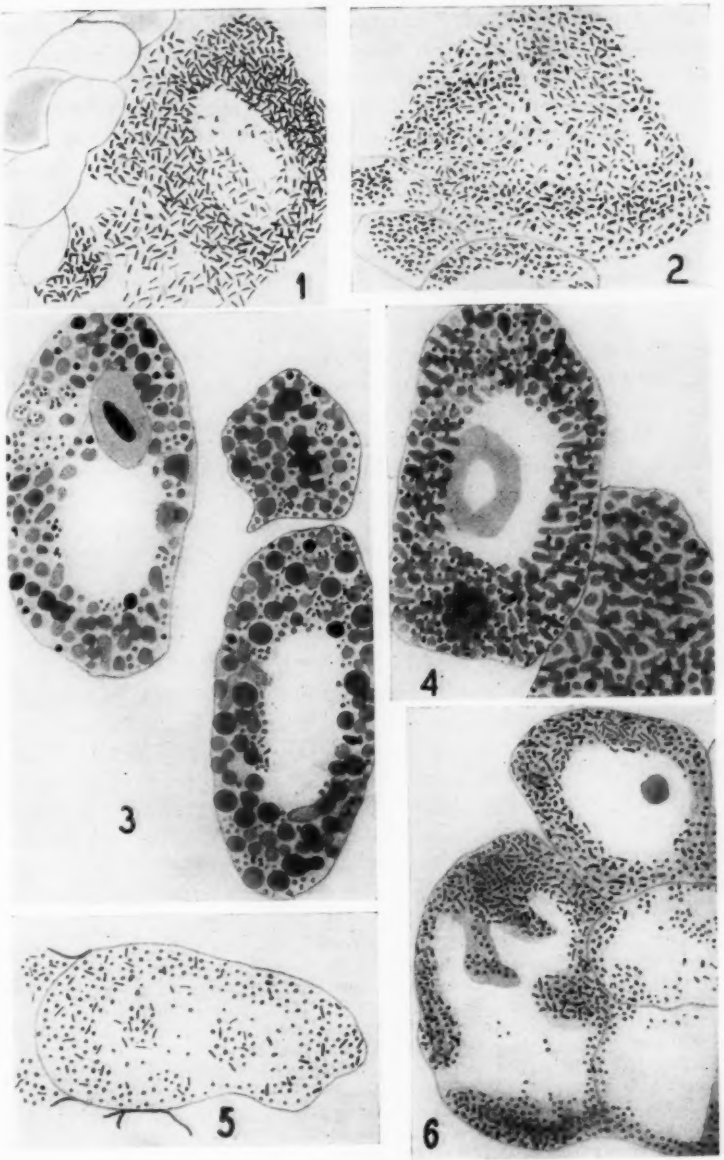

PLATE I

work, a relatively small percentage of the cells examined presented granules or rods suggestive of mitochondria. More rarely, in comparison, have there been cases of cells possessing the larger metamorphic bacterial forms and at the same time exhibiting minute rods or granules, or both, suggesting mitochondria. In some cases where this latter condition has prevailed the minute forms have shown differential staining properties (Plate I, Fig. 3 and Plate II, Fig. 8).

Nodules which had been treated with 95 per cent alcohol or ether for 12 to 24 hours before fixation, or with Altmann's plus 5 per cent of acetic acid, have shown minute granules and rods, sometimes in relatively large numbers. Many of these inclusions stain red with anilin-fuchsin and toluidin blue. Like those appearing in nodules which had not been subjected to the above-mentioned treatment before fixation, they vary to some extent in size, sometimes in the same cell. Figures 9, 10 and 11 (Plate II) illustrate results obtained in different nodules which had been treated in 95 per cent alcohol for 12 to 16 hours and fixed in Murray's fluid and stained with anilin-fuchsin and toluidin blue. Granules and rods can be observed scattered more or less uniformly throughout the cell. While some of these inclusions are stained faintly red and others are almost completely overshadowed by numerous large bacteria, still others can be observed much more readily.

In Fig. 12 (Plate II) a cell containing granules and rods stained red can be observed and illustrates one of many cases following treatment with 95 per cent alcohol for 12 hours and fixed in Altmann's fluid. It will be noted that the cell is completely filled with minute bacteria-like forms and that there is a close likeness between the forms that stain red and those that stain green. Figure 14 (Plate II), and Fig. 15 (Plate III) are drawings made from two separate nodules which were treated with ether for 12 hours before fixing with Champy-Kull's fluid and stained in anilin-fuchsin and methyl green. These two specimens show much variation in number, form and arrangement of granules and rods.

PLATE II

FIGS. 7 and 8. Cells from different nodules, showing differential staining of minute granules. Flemming's and Altmann's fixation, respectively.

FIG. 9. Cell from nodule after 12 hours treatment with 95 per cent alcohol before fixation. Murray's fixation.

FIGS. 10 and 11. Cells from different nodules after 16 hours treatment with 95 per cent alcohol before fixation. Murray's fixation.

FIG. 12. Cell from a section of a nodule after treatment with 95 per cent alcohol before fixation. Altmann's fixation.

FIG. 13. Section from nodule, showing minute granules. Altmann's plus 5 per cent of acetic acid fixation.

FIG. 14. Section of nodule after 12 hours treatment with ether before fixation. Champy-Kull's fixation.

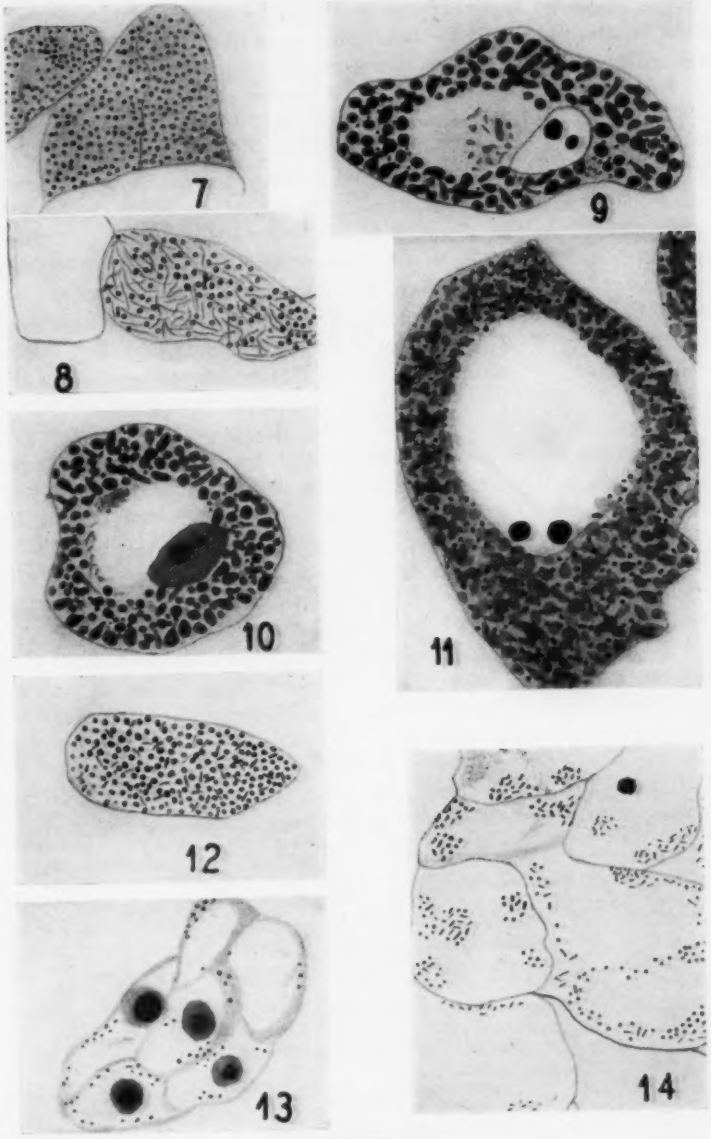

PLATE II

Figure 13 (Plate II) represents a specimen from a nodule which was fixed in Altmann's fluid to which 5 per cent of acetic acid was added, and stained with anilin-fuchsin and methyl green.

In order that a more satisfactory comparison between alcohol and non-alcohol-treated specimens could be made, nodules were cut and the two halves subjected to different treatment. This procedure overcame, in a measure, the difficulties encountered when examining different nodules. Sections of parts of a single nodule mounted on slides in close juxtaposition facilitated a detailed comparative study. Likewise, there remained slight opportunity for variations in fixing and staining procedure. Such a comparison showed a strikingly close resemblance in the rod-like and granular cell contents. In numerous cases there were no observable differences in the size, general arrangement, nor in staining qualities of these inclusions in the separate halves (Plate III, Figs. 16 and 17).

In smears of the nodule, prepared by thorough maceration and allowing them to dry before fixation, one is frequently able to observe minute rods and granules, sometimes in abundance, stained red with anilin-fuchsin and toluidin blue, or methyl green (Plate III, Figs. 18, 19, and 20). The same is true if the smear has been treated with alcohol or ether prior to mitochondrial fixation (Plate III, Fig. 22). Similar forms of bacteria possessing identical staining qualities occur on artificial media which had been inoculated with substance from the nodule or from the soil. Frequently, these minute bacteria from artificial media stain red while larger forms occurring in the same media stain blue or green (Plate III, Figs. 21, 23, and 24).

PLATE III

FIG. 15. Section of a nodule different from the one shown in Fig. 14 after 12 hours treatment with ether before fixation. Champy-Kull's fixation.

FIG. 16 (*a*) and (*b*). Cells from approximately the same region of the same nodule: (*a*) after 12 hours treatment with 95 per cent alcohol before fixation, and (*b*) without alcohol treatment. Murray's fixation.

FIG. 17 (*a*) and (*b*). Cells from approximately the same region of the same nodule: (*a*) after 12 hours treatment with 95 per cent alcohol before fixation, and (*b*) without alcohol treatment. Champy-Kull's fixation.

FIG. 18. Bacteria from nodule smear. Schaudinn's fixation and stained in iron-hematoxylin.

FIG. 19. Bacteria from smear made from a nodule. Altmann's fixation.

FIG. 20. Bacteria from nodule smear. Champy-Kull's fixation.

FIGS. 21 and 23. Bacteria from different nodules and cultured on lithium-agar plate. Altmann's fixation.

FIG. 22. Bacteria from nodule smear, dried and treated with 95 per cent alcohol. Altmann's fixation.

FIG. 24. Bacteria from mannite medium inoculated from the soil. Champy-Kull's fixation.

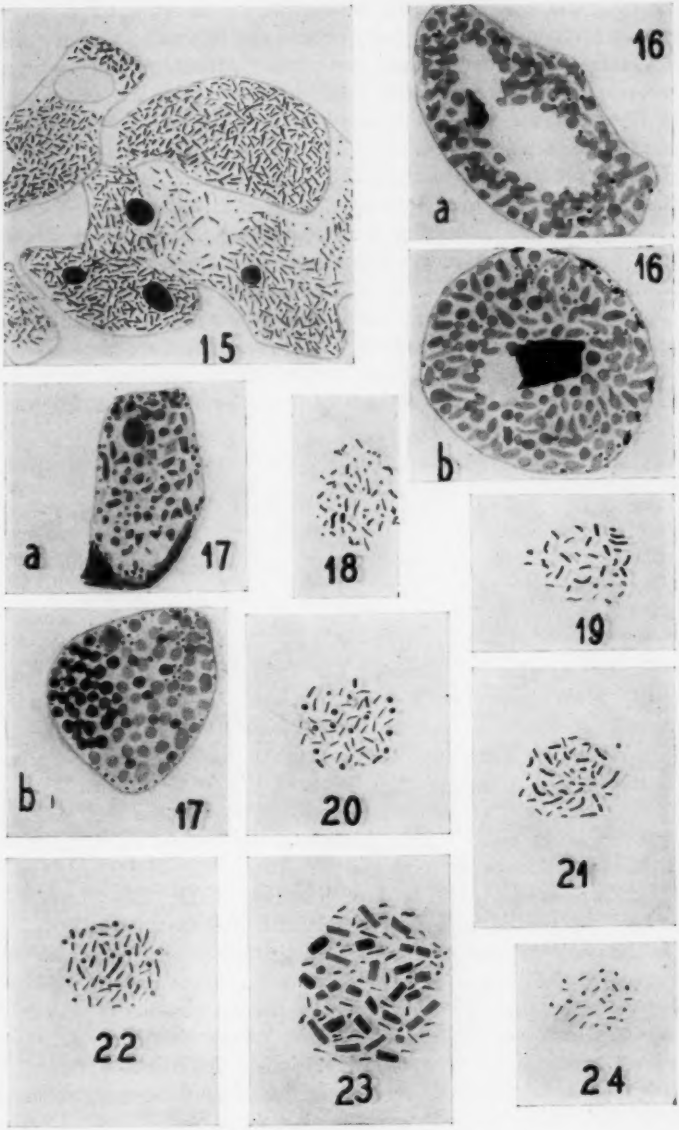

PLATE III

Freshly macerated nodules, in addition to showing large, mature forms of bacteria, also show many minute rod-like and granular forms. Many, due to their minute size, presented a dark appearance. Supravital tests on this material with Janus green revealed nothing definite. There were no indications that the number of dark staining rods and granules had been increased.

Worthy of note at this point is the fact that among the different mitochondrial methods of technique, Regaud's method gave the least satisfactory results, following treatment of the nodule with alcohol. While granules and a few rod-like inclusions frequently appeared in cells after alcohol treatment, they did not stain well nor stand out nearly so clearly as after other methods of fixation nor in cells which had not been subjected to alcohol. There were some indications that granular material in the alcohol-treated nodules represented merely precipitate, and, for that reason have been omitted from the list of illustrations.

DISCUSSION

Topley and Wilson (1932, p. 315) describe the nodule organism, *Rhizobium radicicola,* as one having a life-cycle with gross changes in morphology: "Shows non-motile coccoid forms; very small, highly motile, ellipsoidal form (swarmer), 0.9×0.2 microns; motile rods $2\text{–}3 \times 0.5$ microns." They state with respect to the smaller forms, p. 314: "They are so small as to be able to pass through a Chamberland filter." Löhnis (1921), (Plate 15, Fig. 195; Plate E, Fig. 16; Plate R, Fig. 83), gives an excellent idea of the minuteness and variation in size of rod-like bacteria belonging to *B. radicicola.* It has been thought by some investigators that other microörganisms, besides *B. radicicola,* may enter the nodule of the clover. To quote Wallin (1927), p. 90: "In the young root nodules no other organisms but the *Bacillus radicicola* are present in the cells. As the nodules mature, other microörganisms begin to invade the cells of the nodule. In an old nodule, very few if any *Bacillus radicicola* can be found in the nodule. They appear to have been replaced by a variety of parasitic microörganisms."

In the preceding pages I have shown that minute bacteria, both rod-like and granular, present in the nodule and in the soil are not unlike, in their general appearance and staining properties, rod-like inclusions of the clover nodule and described heretofore as mitochondria. Likewise, the illustrations show how mitochondria-like inclusions appear frequently in nodules which have been subjected to prolonged treatment with alcohol, ether, or fixatives containing acetic acid. Since, in addition, minute bacterial forms of *B. radicicola* enter the clover rootlets and

subsequently bring about the formation of the nodule, together with the probability that still other bacterial microörganisms invade the nodule, it appears likely that the minute inclusions having a mitochondrial appearance represent bacteria. For the fact that among nodules, or halves of nodules, which have received different treatment prior to fixation with mitochondrial fixatives (excepting perhaps Regaud's), there have existed in many cases no observable differences in the nature of minute cell inclusions, strongly indicates that inclusions lacking essential mitochondrial characteristics are present in the nodules of the clover.

A close study of the illustrations given by Cowdry, showing the morphological forms of *B. radicicola* as they occur in the clover nodule, leads me to believe that the minutest of the bacterial forms, such as I have observed in smears of nodules, freshly macerated nodules, as well as from cultures of bacteria from the nodule and from the soil, have not been included in these illustrations as bacteria, but as mitochondria. The reason for such an omission, if true, appears to lie in the fact, largely, that Cowdry is of the opinion that the smallest of bacterial forms found in the nodule stain green with methyl green after mitochondrial fixatives and not red with anilin-fuchsin. He states, p. 340: "A little farther up the rootlet small forms of *B. radicicola* are encountered, as represented in figure 3. They are distinguishable from the mitochondria in the preparations by their green color, their larger size, and their variable distribution in clumps in different cells." As has already been shown in preceding pages and the illustrations, bacteria equaling in size apparent mitochondria as observed in nodules, occur in nodules and in the soil and stain red with anilin-fuchsin and methyl green or toluidin blue. Many of these bacteria are apparently smaller than many red colored rods and granules observed in nodules which may or may not have been treated with lipoid solvents before fixation.

For the reason that differential staining sometimes occurs among these inclusions, whether in the nodule, smears, or on slides prepared from cultures of material from the nodule and from the soil, it would appear that the slightest variation in the chemical composition of the inclusions, or in technique, is often all that is necessary to alter the staining reaction of these minute forms. For the fact that, in numerous cases, where nodules have been subjected to alcohol treatment, cells occur which are completely filled, or nearly so, with minute rods and granules, some of which stain red while others stain green, makes it quite obvious that minute bacteria within the cell of the nodule may stain like mitochondria.

From the above observations it would seem that there are three pos-

sible conclusions. Firstly, either mitochondria as such do not exist in these nodules, but have been confused with an "invading form" of minute bacteria from the soil; or secondly, mitochondria possess characteristics which have not been fully recognized, in that they may withstand alcohol, ether, dilute acetic acid, maceration of the nodule, and drying; or thirdly, they are indistinguishable in the nodule from minute forms of bacteria with our present-day methods of technique. Certainly, the above methods of technique have aided little in making a clear distinction between bacteria and mitochondria, but in the case of the clover nodule the results materially strengthen the indications that the alleged mitochondria are merely forms of minute bacteria.

CONCLUSIONS

1. The morphology of minute cytoplasmic inclusions as they occur in the nodule of the clover, their response to Janus green and lipoid solvents, staining reaction, fail to produce sufficient evidence for placing them in the category of mitochondria.

2. Bacillary and granular bacteria occur in clover nodules, in nodule smears, in cultures of material from nodules and from the soil on artificial media, which possess staining qualities and physical features characteristic of mitochondria.

3. Minute rods and granules, having the physico-chemical properties of mitochondria, can be demonstrated in some nodules which have been subjected to prolonged treatment with 95 per cent alcohol, ether and fixatives containing 5 per cent of acetic acid.

4. Differential staining cannot be relied upon in this case to distinguish bacteria from the alleged mitochondria, since certain forms of minute bacteria found in nodule-smears and cultures made from the nodule and from the soil stain red with anilin-fuchsin and methyl green after mitochondrial fixatives, just as do many rod-like and granular inclusions in sectioned material.

5. Indications are that the slightest variation in technical manipulations, or possibly chemical composition of different regions of the nodule, is often sufficient to alter the staining reaction of otherwise identical minute forms occurring in the clover nodule.

6. Janus green apparently does not stain any of the minute rods nor granules found in freshly macerated nodules.

7. The results of this investigation appear to support strongly the inference that minute bacterial forms have heretofore been misinterpreted as mitochondria in the nodule of the clover.

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THE RELATION BETWEEN LUMINOUS INTENSITY, ADAPTATION TO LIGHT, AND RATE OF LOCOMOTION IN AMOEBA PROTEUS (LEIDY)

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Introduction

Strasburger (1878), Loeb (1890), and Davenport (1897) maintain that organisms appear to move faster in strong light than in weak light because their path is straighter and orientation is more precise and they conclude that the rate of locomotion is independent of intensity. This conclusion is supported by the results obtained by Mast (1910, 1911) on *Amœba proteus* and blow-fly larvæ, Dolley (1917) on *Vanessa antiopa*, Minnich (1919) on *Apis mellifera*, Mast and Gover (1922) on *Phacus pleuronectes* and *Euglena gracilis*, Folger (1925) on *Amoeba proteus*, and Ullyott (1936) on *Dendrocœlum lacteum*.

But it is not supported by those obtained by Oltmanns (1892), Holmes (1903), and Laurens and Hooker (1920) on *Volvox*, Yerkes (1903) on *Gonionemus*, Herms (1911) on blow-fly larvæ, Patten (1917) on the whip-tail scorpion, Buddenbrock (1920) on *Helix*, Moore and Cole (1921) on the Japanese beetle and *Drosophila melanogaster*, Clark (1928) on *Dineutes assimilis*, and Welsh (1932) on *Unionicola*.

Clark made a more thorough study of the problem than any of the other investigators. He found that with increase of intensity, the rate of locomotion increases to a maximum at 0.5 m.c., then remains constant until 8,000 m.c. is reached, and then decreases. Trezise (1936) obtained similar results in observations on *Dineutes nigroir*.

Mast (1907, 1927) contends that the activity of organisms in any given luminous intensity is closely correlated with the state of adaptation of the organism to that intensity. This contention is supported by results obtained by Walter (1907) in observations on *Planaria gonocephalus*. He maintains that this organism moves faster in light than in darkness, but the results which he presented show that if the specimens are transferred from darkness to light and left there, the rate of locomotion decreases so that after 10 minutes it is much lower than it was in darkness.

It is consequently obvious that there is great diversity in the results obtained by different investigators concerning the relation between rate of locomotion and luminous intensity. This is doubtless largely due to the fact that in most of the investigations the state of adaptation was not adequately considered.

Material and Methods

Amoeba proteus (Leidy) was used exclusively in the following experiments. It was raised in finger-bowls containing a few grains of

Table I

Relation between rate of locomotion in *Amoeba proteus* and intensity of light. The "total average rate" is the mean for all measurements at the indicated intensities of light. The "mean maximum rate" is the mean for all measurements made after the maximum rate was reached. The time required to reach the maximum rate was obtained by inspection of the graphs for each intensity of light. The standard deviation was calculated for the rate after the maximum was reached.

Intensity	Number of specimens used	Total average rate	Mean maximum rate	Time required to reach maximum	Percentage of measurements on lobose forms	Standard deviation
meter candles		*micra/min.*	*micra/min.*	*minutes*		*micra/min.*
50	21	128.8	128.8	1	7.4	10.8
225	18	124.8	131.0	11	8.7	9.0
4,170	14	145.8	147.4	16	16.5	8.6
7,530	16	160.0	186.6	14	9.0	11.3
11,120	18	176.2	198.4	11	12.0	9.3
13,140	18	191.3	215.7	15	10.8	6.4
15,000	19	198.1	219.3	8	16.8	11.4
17,000	20	176.0	211.1	17	10.8	11.4
20,000	23	170.0	200.2	22	14.7	4.3
24,000	22	160.9	205.7	23	12.4	14.2
26,600	21	151.8	195.6	22	17.2	9.8
40,000	23	120.7	150.2	30	20.4	8.5

rice and numerous chilomonads in Hahnert solution, which consists of pure water (1 liter), $CaCl_2$ (4 mg.), $NaHCO_3$ (4 mg.), $CaH_4(PO_4)_2$ (2 mg.), KCl (4 mg.), $Mg_3(PO_4)_2$ (2 mg.). The cultures were kept at a temperature of 24°–26° and at a luminous intensity of about 20 meter-candles.

All the experiments were performed in a darkroom. The light used was produced by a monoplane filament stereopticon bulb mounted in a projection lantern which was in turn enclosed in a box, light-tight, except for an opening 25 cm. square. A parallel-sided glass vessel 8 cm.

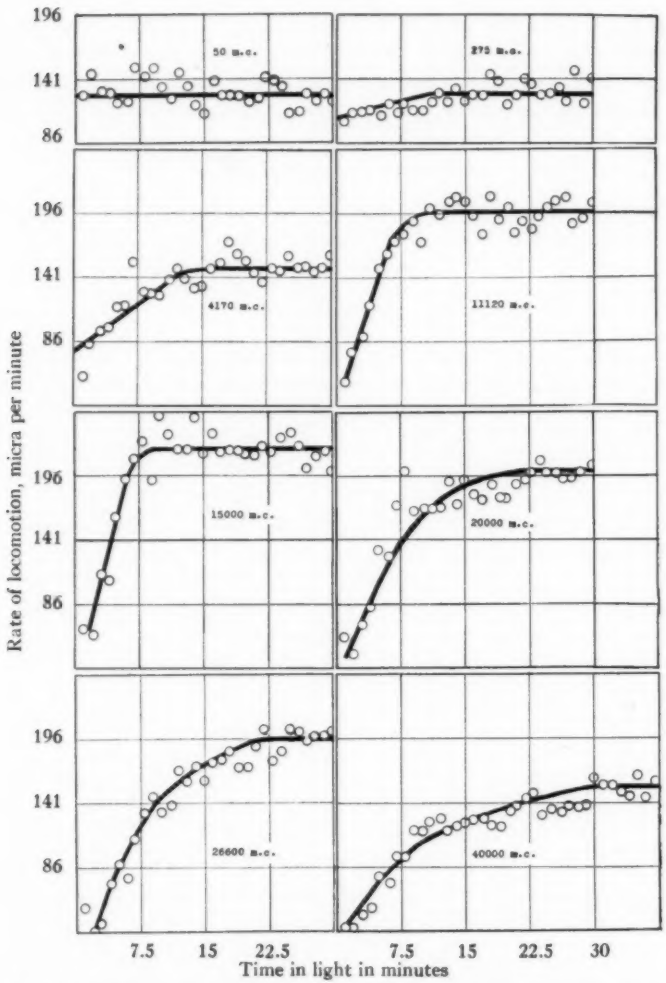

FIG. 1. Relation between adaptation to light of different intensities and rate of locomotion in *Amoeba proteus*.

Each point in the figures represents the average for one measurement on each of 14 to 23 specimens (see Table I). The time in light is the time from the beginning of movement after exposure until the measurements were made.

thick, filled with distilled water, was placed in the box in front of the lamp, to absorb the heat waves.

Three stereopticon bulbs were used (300, 500, and 1,000 W., 120 V.). The voltage on the line was maintained at 110 ± 2 volts by means of a rheostat. The desired intensities of light were obtained by means of these bulbs and Eastman Kodak neutral-tint filters.

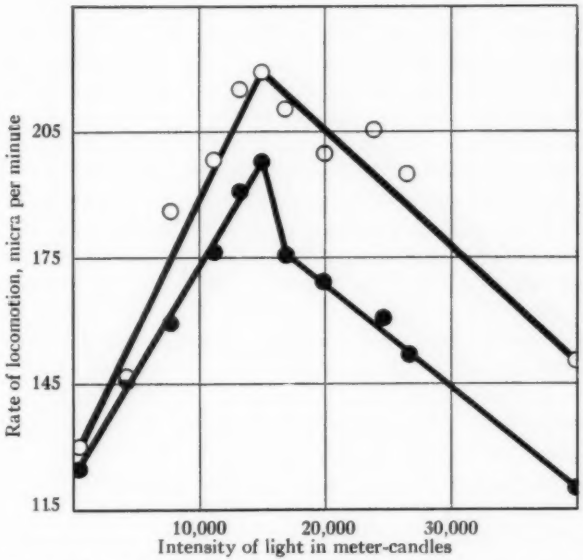

FIG. 2. Relation between intensity of light and rate of locomotion in *Amoeba proteus*.

Upper curve, mean maximum rate; lower curve, total average rate.

Each point on the lower curve is the average for 420 to 920 measurements on 14 to 23 specimens. Each point on the upper curve represents the average for the same number of measurements in the lowest intensity but progressively fewer as the intensity increases (see Table I and Fig. 1).

The beam of light was reflected up through the stage of a compound microscope by means of the substage mirror. The microscope had no substage condenser. The intensity on the stage was measured directly by means of a Weston photometer.

A Pitts warming stage (Pitts, 1932) was fastened to the stage of the microscope. This consists essentially of a beaker in a metal box so arranged that water of any desired temperature can be passed through

the box and around and under the beaker.[1] The bottom of the beaker consisted of smooth Pyrex glass.

The observations were made as follows: A large number of amœbæ were taken from a vigorous culture and put into a small glass dish containing 10 cc. redistilled water and left ten minutes. The water was then changed four times. Then about 60 large specimens with few food vacuoles were selected and put into a dish containing redistilled water and left without food 15 hours or longer in darkness at a temperature of 24° to 26°. Then 5 to 10 of the specimens were transferred to a dish containing .001 N $CaCl_2$ and immediately transferred again, with $CaCl_2$ solution, to a vaseline enclosure on the bottom of the beaker in the Pitts warming stage and covered with a cover-glass. The solution in the enclosure was so deep that the amœbæ did not touch the cover-glass. The temperature in the beaker was maintained at $24 \pm 0.5°$.

They were then subjected to light of 50 m.c. for five minutes, then to darkness for 30 minutes. Then a weak red light was turned on and a specimen which was monopodal or nearly so was selected, brought to focus, and exposed to white light of the desired intensity, from the projection lantern.

The posterior end of the image of the amœba, projected on black paper by means of a camera lucida, was then outlined with a yellow pencil, once a minute for 30 or 40 minutes, and the rate of locomotion calculated. If the specimen under observation came in contact with other specimens or with the vaseline ring during this time the record was discarded. The whole process was then repeated with other specimens in light ranging from 50 to 40,000 meter candles. The results obtained are presented in the following paragraphs, Table I, and Figs. 1 and 2.

Results

In all but the lowest intensities movement ceased shortly after the specimens were exposed, and in the highest intensities it did not begin again until 2–7 minutes later. In these intensities movement usually began in a pseudopod on the upper surface of the amœba. This pseudopod often extended until it contained nearly all the substance in the amœba, then a pseudopod formed at the base and extended in contact with the slide. This frequently occurred several times during the process of adaptation to light.

In the lower intensities the specimens were fairly consistently and firmly attached to the substratum and monopodal in form. In the

[1] Small pieces of glass 5 mm. thick were cemented to the inner base of the warming stage so that a constant depth of water under the beaker obtained.

higher intensities they were much less consistently monopodal and attachment to the substratum was more sporadic.

Figure 1 shows that, in all except the lowest intensity, as the time of exposure to light increased, the rate of locomotion rapidly increased to a maximum and then remained constant; and that, as the intensity increased, the time required for the rate to reach maximum decreased from about 11 minutes at 225 m.c. to a minimum of about 8 minutes at 15,000 m.c., and then increased to about 30 minutes at 4,000 m.c. This proves that the rate of locomotion in *Amoeba proteus* is closely correlated with the state of adaptation to light and it indicates that, except in the very low intensities, the time required for light-adaptation is least at 15,000 m.c.

Table I and Fig. 2 show that as the intensity to which the amœbæ were exposed increased, the rate of locomotion after the amœbæ were fully light-adapted, increased from 128.8 ± 10.8 micra per minute at 50 m.c. to a maximum of 219.3 ± 11.4 micra per minute at 15,000 m.c., and then decreased to 150.2 ± 8.5 micra per minute at 40,000 m.c. This proves that the rate of locomotion in *Amoeba proteus* is very definitely correlated with the intensity of the light, and that the optimum intensity of light is 15,000 m.c. Figure 2 shows also that the total average rate is correlated with the intensity of the light in essentially the same way as the mean maximum rate.

Table I shows that the percentage of lobose forms increased with increase in luminous intensity, and that the standard deviation for the mean maximum rate is relatively low.

The average rate of locomotion of 20 amœbæ for 30 minutes each in the red light used in the experiments described above, was 134.7 ± 8.9 micra per minute. There was no indication of decrease in rate in any of the specimens after exposure to the red light. The intensity of the red light was not measured but the rate of locomotion in it was somewhat higher than the rate of locomotion in white light of 225 m.c. in which there was marked retardation (Fig. 1). The facts that the rate in red light (in which there was no retardation) was higher than the rate in white light (in which there was marked retardation) and that blue is very much more efficient than red in inducing retardation (Harrington and Leaming, 1900 and Mast, 1910) strongly indicate that retardation and decrease in rate of locomotion with increase in intensity (in the higher intensities) observed in white light, is due largely to the action of blue and other short waves, and that increase in rate of locomotion with increase in intensity (in the lower intensities) is due largely to the action of red and other long waves.

There is no evidence which indicates the nature of the action of the longer waves of light, but it is well known that the shorter waves tend to induce gelation. It is therefore highly probable that retardation in rate of movement is due to the gelating effect of these waves.

Summary

1. The rate of locomotion in *Amoeba proteus* is definitely correlated with the intensity of the light to which it is exposed and the state of adaptation.

2. In light of any given constant intensity, as adaptation to light increases, the rate of locomotion increases to a maximum and then remains constant, but in constant light of different intensities, the time required for adaptation decreases from about 15 minutes at 225 m.c. to a minimum of about 7 minutes at 15,000 m.c. and then increases to about 30 minutes at 40,000 m.c. and the rate of locomotion increases from 128.8 ± 10.8 micra per minute at 50 m.c., to a maximum of 219.3 ± 11.4 micra per minute at 15,000 m.c., and then decreases to 150.2 ± 8.5 micra per minute at 40,000 m.c.

3. Increase in rate of locomotion with increase in intensity to optimum at 15,000 m.c. is largely due to some unknown action of the longer waves of light. Decrease in rate with increase in intensity beyond the optimum is probably due to the gelating action of the shorter waves of light.

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THE RÔLE OF THE HYPOPHYSEAL MELANOPHORE HORMONE IN THE CHROMATIC PHYSIOLOGY OF FUNDULUS[1]

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The endocrine control of melanophore reactions of vertebrates has been extended within the past twenty years to the majority of animals which exhibit color changes. It has long been generally conceded that the pituitary gland is the sole regulator of chromatic activity in the Amphibia (Smith, 1916; Allen, 1916; Atwell, 1919; Swingle, 1921; Hogben, 1924). Within recent years, the cyclostomes (Young, 1935), the elasmobranchs (Lundstrom and Bard, 1932), and certain reptiles (Noble and Bradley, 1933; Kleinholz, 1936) have been found to react to hypophysectomy by complete pallor due to the concentration of the pigment in the cutaneous melanophores. It is also generally acknowledged that such hypophysectomized specimens remain indefinitely pale regardless of certain environmental conditions which in normal specimens would induce melanophore expansion. Furthermore, these hypophysectomized specimens cannot normally exhibit the dark phase of their coloration unless the melanophore hormone of the pituitary is administered in one form or another to them.

The teleosts are, as far as I am aware, the only color-changing vertebrates thus far investigated which do not behave to hypophysectomy like those already mentioned. The melanophores of the teleosts have long been known to be controlled by the sympathetic nervous system (von Frisch, 1911), but the importance of the pituitary gland has not been satisfactorily evaluated except perhaps in one case, *Ameiurus* (Parker, 1934*a*; Abramowitz, 1936).[2] In *Fundulus*, the relation between the pituitary and the chromatic function is uncertain. Parker (1935) is of the opinion that the hypophysis is without functional significance as a normal means of controlling color change in *Fundulus*, although later (1936) he writes that certain responses call for further

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[2] Recently Veil (1937) has reported that *Ameiurus* reacts to hypophysectomy as the selachians do. Her paper is probably a preliminary report since very little or no data are given in support of this conclusion.

elucidation. This paper is intended to define the rôle of the hypophyseal melanophore-expanding hormone in the chromatic physiology of *Fundulus.*

Desmond (1924) was apparently the first to investigate the effect of the pituitary on the melanophore responses of *Fundulus,* but his work has been overlooked by the subsequent investigators of this problem. He found that hypophysectomy was without effect on the melanophore reactions of young *Fundulus.* This negative effect following hypophysectomy could not be attributed to the absence of the melanophore hormone in the pituitary of *Fundulus,* for he found that while pituitary transplants from adult fish or amphibians into *Fundulus* were without effect on the melanophores of the host, similar transplants made into hypophysectomized axolotls or tadpoles produced the typical darkening of these hosts. It thus appeared that the pituitary of *Fundulus* contained the melanophore-expanding hormone which was effective on amphibian melanophores but ineffective on its own black pigment cells. Matthews (1933) also observed that hypophysectomy did not affect the melanophores of adult *Fundulus.* The melanophores expanded normally when pituitaryless specimens were adapted to a black background and contracted promptly when adapted to a white background. However, when Matthews immersed isolated scales in a water extract of *Fundulus* pituitaries, the scale melanophores always contracted. On the belief that the pituitary contained a melanophore-contracting hormone, Matthews reasoned that it might conceivably affect the contraction of denervated melanophores in a caudal band (Mills, 1932). However, denervated trunk or tail areas faded in the same time in hypophysectomized as in intact animals during prolonged white-background adaptation.

Thus the situation became complicated, for Matthews' work indicated that a contracting hormone was present in the pituitary of *Fundulus,* and yet the melanophores, innervated or denervated, contracted normally in the absence of the pituitary. The presence of a contracting principle was disputed by Kleinholz (1935), who showed that *Fundulus* pituitaries could darken normal white-adapted frogs and catfishes, and hypophysectomized lizards, and furthermore that injections of pituitrin or extracts of crushed *Fundulus* pituitaries were followed by a darkening of a pale denervated caudal band in normal white-adapted *Fundulus.* The latter experiment is significant in relation to the neurohumoral theory (Parker, 1932) for it raises the possibility that the black background response (the expansion of contracted denervated melanophores in a normal *Fundulus* when adapted to a black background) might be due, not to a dispersing neurohumor as Parker

(1934*b*) has maintained, but to the melanophore-expanding hormone of the pituitary (Kleinholz, 1935).

The responses of teleost melanophores to various hypophyseal extracts are by no means clarified. Spaeth (1917), Hewer (1926), Odiorne (1933) and Matthews (1933) have reported melanophore contraction following treatment with certain hypophyseal extracts. The significance of these reports is enhanced by Hogben and Slome's (1936) recent work with amphibians where, in *Xenopus*, evidence for a dual pituitary control by melanophore-expanding ("black") and contracting ("white") hormones has been accumulated. Thus it may be possible that the pituitary gland of *Fundulus* contains a contracting melanophore principle. The existence of a melanophore-expanding hormone in the teleost pituitary rests on a more substantial basis, for this principle has been shown to be present in the pituitary glands of all chordates from cyclostomes through the primates, including man. The complexity of melanophore responses to pituitary extracts was increased by the work of Przibram (1932), who showed that the injected dosage was a factor determining the direction of melanophore response. He found that low dosages of a commercial pituitary extract resulted in an expansion of amphibian melanophores, while when higher dosages were employed the melanophores contracted.[3] This result he offered as an explanation of the conflicting reports of the effect of pituitary extracts upon the melanophores of the same fish (*Phoxinus*) by Abolin (1925) and by Hewer (1926). There is thus a great difference of opinion regarding the responses of teleost melanophores (intact, isolated from the body, or isolated experimentally—denervated—within the body) to various pituitary extracts.

Methods

Large healthy specimens of *Fundulus heteroclitus* were used throughout this work. A stock supply of fish was maintained in a large aquarium of running sea water and fed daily with generous portions of macerated clams and live shrimp. Background responses were elicited by the customary white or black vessels, three of each kind being placed

[3] Przibram's experiments were carried out with a commercial pituitary extract (Infundin), which is not primarily prepared for the melanophore hormone and which is probably contaminated with preservatives, and other posterior pituitary hormones. When high concentrations of Infundin were employed, the presence of the extraneous substances might have conceivably complicated the response. My results with hypophysectomized frogs do not substantiate Przibram's explanation for many such specimens injected with high dosages of a relatively pure melanophore hormone (1 gram equivalent of whole sheep pituitary powder potentiated 25 times by alkalinization) always became black and remained so for two weeks.

on a lead table and arranged so that a constant current of sea water flowed through all the dishes. All six vessels were illuminated by two 60-watt lamps. Hypophysectomies were performed by the opercular approach (Abramowitz, 1937). Slightly over 200 hypophysectomies were performed and an equal number of normal specimens kept as controls. Since the operator can determine whether the entire gland is being removed, histological examination of the hypothalamus was deemed unnecessary. When the gland was seen to fragment during an operation, the animal was immediately discarded. Twelve hypophysectomized *Fundulus* were selected at random and the region of the hypothalamus dissected under an operating binocular but no trace of pituitary tissue could be found. Denervating operations in the base of the tail were carried out by Wyman's technique (1924). The width of these bands was in every case 2 mm., since all cuts were made with the same instrument. The technique of extracting the melanophore-expanding hormone from the blood was somewhat similar to that described by Jores (1933) for man.

Results

I. Effect of Hypophysectomy on the Responses of Denervated Melanophores to Backgrounds

A denervated band of melanophores was established in the base of the tail of each of 26 *Fundulus*. Twelve of these specimens were hypophysectomized, and these and the remaining 14 unoperated animals placed in illuminated white vessels. Three days later the denervated bands which during this time appear black due to melanophore expansion become completely pale (melanophore contraction) in both unoperated and hypophysectomized groups. The pituitary then does not affect the contraction of denervated melanophores during prolonged white background adaptation immediately following denervation, as first demonstrated by Matthews (1933). Then, both sets were transferred to an illuminated black background. Twelve hours later, 11 of the 14 normal animals exhibited fully dark bands; the remaining 3 showed partly dark bands. In the hypophysectomized series, 6 showed faintly dark bands and 5 exhibited completely pale bands.[4] After 36 hours of black-background adaptation, there was no change in either of the two series of animals.

[4] In all of the experiments cited, microscopical examination of the melanophores was made. In the absence of a quantitative treatment of melanophore responses, the following descriptive terms will be employed: fully dark—complete melanophore expansion; partly dark—slightly more expanded than intermediate; faintly dark—melanophores more contracted than intermediate; pale—complete melanophore contraction.

Thus the pituitary seems to be indispensable for the complete expansion of denervated melanophores following black-background adaptation. Since this experiment is important from the standpoint of the neurohumoral theory (Parker, 1932) it was repeated in different ways. Nineteen *Fundulus* were hypophysectomized, and denervating operations induced in their caudal fins. These were placed on a white background for 13 days. By this time, the cut nerves in the tail fin have completely degenerated (Parker and Porter, 1933; Abramowitz, 1935). The bands are also completely pale due to extreme melanophore contraction. Thirteen additional animals to serve as control were treated in the same way except that they were not hypophysectomized. At the end of 13 days, both sets were transferred to a black background for 24 hours. The control animals showed fully black bands but in the hypophysectomized group, only 1 band was fully black, 12 were faintly dark, and 6 completely pale.

This black-background experiment was again repeated in a third manner. In 30 *Fundulus,* caudal bands were established and the animals white-adapted for 5 days. Ten animals were then changed to a black background for 24 hours. The remaining twenty were hypophysectomized and also transferred to a black background for 24 hours. All 10 normal animals exhibited fully dark bands. In the hypophysectomized group, 11 showed faintly dark bands, 1 fully dark, and 8 completely pale. The experiment was continued, and the specimens examined after 40 hours of black-background adaptation. The normal group still showed fully dark bands. Half of the hypophysectomized evidenced faintly dark bands, and half showed completely pale bands.

Thus, it appears that the melanophore (expanding) hormone of the pituitary is necessary for complete expansion of denervated contracted melanophores in the change from white to black background. In this respect, it is in agreement with *Ameiurus* (Abramowitz, 1936). So far nothing has been said of the reactions of the innervated melanophores following hypophysectomy. In all of the experiments, the innervated melanophores contracted promptly when the fish was exposed to a white background in both normal and hypophysectomized. Both sets of animals also showed expansion of the innervated melanophores when exposed to a black background. These statements are confirmatory of Matthews' observations. However, my hypophysectomized animals never became quite as dark as normal animals when exposed for equal periods of time to a black environment.

These experiments, as well as those of Matthews, indicate that the hypophysis has no effect on the contraction of either innervated or denervated melanophores. The latter must be due to some factor

(neurohumoral) other than nervous action or a possible hypophyseal melanophore-contracting hormone. The full expansion of contracted denervated cells, however, does not occur in the absence of the melanophore-expanding hormone, and the expansion of the innervated melanophores is only slightly retarded in hypophysectomized animals. It would seem, therefore, that Parker's conception of the neurohumoral theory as applied to *Fundulus* may be modified to include the aid of the melanophore-expanding hormone of the pituitary.

II. Presence of the Melanophore (Expanding) Hormone in the Blood of Fundulus

If these results are correct it should be possible to obtain the melanophore hormone from the blood of *Fundulus,* especially the black-adapted specimens. Previous attempts to detect the presence of hormones in the blood of *Fundulus* have been unsuccessful. Mills (1932) injected blood from a dark *Fundulus* into a pale one, but without effect on the melanophores of the latter. The reverse experiment, blood from a pale fish injected into a dark one, was equally negative. Parker (1934*b*) also reported similar experiments and similar negative results. Obviously, these experiments indicate that the blood of a dark *Fundulus* is ineffective in eliciting expansion of the innervated melanophores of a pale animal, but they do not prove that the blood of *Fundulus* does not contain the melanophore-expanding hormone. The fact that blood of a dark fish does not cause a pale fish to darken is due, as shown in the next section, to the insensitivity of the innervated melanophores of *Fundulus* to the melanophore hormone. Consequently, the presence of the latter could not be demonstrated by the experiments already cited.

The test was therefore made on the hypophysectomized frog, which in my experience is the most reliably sensitive object for the biological detection of the pituitary melanophore hormone. Specimens of *Rana pipiens* were totally hypophysectomized by a direct oral route, and within the following three hours these specimens became totally pale. Hypophysectomized males (30–40 grams in weight) were used throughout. Blood was drawn from 10 black-adapted specimens and extracted with 10 cc. of 50 per cent ethanol by boiling for 2–3 minutes. The soluble portion was separated by centrifugation, dried, extracted with 70 per cent ethanol. The soluble portion was separated, taken up in 0.5 cc. distilled water, and injected into the dorsal lymph sac of hypophysectomized frogs. These became dark within an hour and remained dark for 5 hours, after which they became again pale. A similar test with the blood of 10 white-adapted fish also gave a positive response

but less intense, inasmuch as the frogs remained dark for 3 hours. Repetition of the experiment with blood from white-adapted hypophysectomized or black-adapted hypophysectomized specimens (hypophysectomized 7 days previously) did not produce any response in the pituitaryless frogs.

III. Quantitative Studies on the Melanophore Hormone of the Pituitary of Fundulus

Having demonstrated the presence of the melanophore-expanding autocoid in the blood, and the inability of the denervated melanophores to expand fully in the absence of this hormone from the circulation, it was necessary to determine quantitatively whether the pituitary could elaborate sufficient hormone to affect the denervated cells. Thirty white-adapted hypophysectomized specimens containing pale denervated bands were injected intraperitoneally in groups of 6 animals, each with various known amounts of melanophore hormone prepared from commercial sheep pituitary powder. All experiments in this section with sheep melanophore hormone were performed with dilutions of the same stock solution. Quantitative treatment is obtained by expressing the injected dosage in terms of gram equivalents of commercial pituitary powder. Fish of equal weight (approximately 10 grams) were chosen and the specimens after injection were, of course, returned to the white background for the duration of the experiment. The minimal dosage necessary to darken a pale band was found to be 1 mg. equivalent. In no case did the innervated cells expand although as much as 1 gram equivalent was injected. Doses higher than this amount were sometimes followed by death, and the usual darkening of the entire animal. Thus the melanophores of a white-adapted fish, maintained in a continually contracted state by efferent motor impulses from the C.N.S., seem to be absolutely insensitive to the melanophore-expanding hormone. Intraperitonal injections of various known dilutions of the same stock solution into hypophysectomized frogs disclosed that the minimal dose necessary to cause the beginning of melanophore expansion in the web of the legs was 0.01 mg. equivalents. The minimal effective dose for the denervated tail melanophores of *Fundulus* is thus 100 times greater than that for the melanophores of the frog. This difference in sensitivity is especially marked when the weights of the two animals are compared (frog 35–40 grams, *Fundulus* 10 grams) and when the numbers of reacting cells are compared. The two-millimeter denervated caudal band contains roughly 7,000 melanophores, while the entire skin of the frog, although no computations were made, contains easily many

times this figure. These experiments seem to explain the negative results of Mills and Parker.

The pituitary of an ordinary sized *Fundulus* (10 cm.) when extracted with distilled water contains approximately four frog units. (We define the frog unit as the amount of hormone contained in 0.2 cc., which when injected into the dorsal lymph sac of hypophysectomized frogs produces a reaction (melanophore expansion) during 3 hours. The period of 3 hours is measured as the time intervening between the time of injection and the time at which the animals regain their condition of pallor.) However, if a pituitary is extracted with a small amount of N/10 NaOH, boiled, and neutralized to pH 7 with N/10 HCl, the gland assays at 100 frog units. The potentiation of the melanophore hormone by alkali is thus about twenty-five fold, in agreement with the results of Stehle (1936). The equivalent of 1 *Fundulus* hypophysis, extracted with distilled water, is ineffective in darkening a pale denervated band of a normal white-adapted animal. However, the band may be made fully dark with the equivalent of $\frac{1}{5}$–$\frac{1}{10}$ of a *Fundulus* pituitary when extracted with alkali. Thus there appears to be sufficient hormone in the pituitary of this fish to aid in the normal process of expansion of denervated melanophores. Similarly, blood of one dark *Fundulus,* when extracted with 50 per cent and 70 per cent ethanol, and then boiled with 1.0 cc. of N/10 NaOH, and neutralized to pH 7 is also effective in evoking a darkening of a pale band in a normal white-adapted fish. Thus, there appears to be sufficient melanophore hormone circulating in the blood of *Fundulus* to establish it as a normal agency in the chromatic physiology of this fish.

Summary

1. After hypophysectomy in *Fundulus,* denervated melanophores cannot exhibit the normal black-background response. Normally innervated melanophores are only slightly affected.

2. The inability of the denervated melanophores in hypophysectomized animals to expand completely is due to the absence of the hypophyseal melanophore hormone from the blood.

3. There is sufficient melanophore hormone in the pituitary and in the circulating blood of *Fundulus* to establish it as a significant agency in the melanophore responses of this teleost.

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THE EFFECT OF ITS NITROGEN CONTENT ON THE DECOMPOSITION OF THE POLYSACCHARIDE EXTRACT OF CHONDRUS CRISPUS

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It has been frequently pointed out (Keys, Christensen and Krogh; Johnson; Waksman and Carey, et al.), that the bacterial decomposition of organic matter in stored sea water is limited by the supply of available nitrogen. A rather striking illustration of this arose from an experiment in which the organic material used was the purified polysaccharide extract of the marine alga, *Chondrus crispus,* which provides as well a natural source of nitrogen.

Method

Oxygen consumption was used as the index of decomposition. The customary procedure of storing glass-stoppered bottles (of approximately 200 ml. capacity) under water, at room temperature (20–25° C.), in the dark, was followed. The oxygen remaining was determined by the Winkler titration method, at various intervals. The results given are averages of at least two bottles, more often of three.

Experimental

In a preliminary experiment, the rate of decomposition of the extract was compared with that of the plant itself. The results, given in Table I, show the whole plant to be more readily decomposed than the extract. This fact was attributed to the lower quantity of nitrogen in the latter. For instance, plants containing 1.20 per cent nitrogen, on a dry weight basis, might yield an extract having only 0.30 per cent of nitrogen (Butler, 1936). The method of preparing the extract was described in a previous paper (Butler, 1934).

Table II shows the effect of various amounts of nitrogen, added as nitrate, on the decomposition of the extract.

It is evident from this table that the decomposition was increased in proportion to the amount of nitrogen added, up to a certain point

[1] Contribution No. 144.

only; other factors then apparently become limiting. In seven days the decomposition occasioned with the addition of 0.042 mgm. of nitrate nitrogen was as great as with 0.140 mgm., at least as measured by oxygen consumption. The fact that, with the lower quantity of nitrate, seven days were required to bring about the same amount of decomposition that was accomplished in three days with the larger quantity, suggests a possibility of the nitrogen being utilized more than once. This, however, is not likely, for von Brand, Rakestraw and Renn (1937) have shown that decomposition stops only after an interval varying

TABLE I

Decomposition of Chondrus crispus and its Polysaccharide Extract

Material	Mg. added per bottle	Oxygen consumed (ml. per liter)				
		1 day	2 days	3 days	5 days	6 days
Sea water only	—	0.09	0.24	0.36	—	0.57
Chondrus	3	1.11	1.71	1.89	—	4.32
"	5	—	1.26	—	3.48	—
"	10	1.38	3.60	4.32	—	5.46*
"	20	—	3.18	—	5.88*	—
Extract	25	0.18	0.54	0.81	—	1.47
"	5	—	0.81	—	1.92	—
"	10	0.66	1.17	1.68	—	3.36
"	20	—	1.68	—	5.31	—

* All oxygen consumed.

from 8–20 days. A more probable explanation is that the bacterial population supported by the smaller quantity of nitrogen has a much lower total metabolism than that of the abundant population when nitrogen is more plentiful.

The bacterial numbers given in Table II were furnished by Dr. Margaret Hotchkiss at the Woods Hole Oceanographic Institution, and are simply included here as a matter of record, but will not be discussed.

Having seen the effect on the breakdown of the *Chondrus* extract of adding various quantities of inorganic nitrogen, the effect of different quantities of organic nitrogen became of interest. Since extracts from different collections of *Chondrus* contain varying amounts of nitrogen (Butler, 1936), it was a relatively simple matter to study the effect of this naturally occurring organic nitrogen on the decomposition of the polysaccharide complex. For the purpose, a series of bottles was prepared in which each group contained the extract from a different monthly collection of *Chondrus* plants. As each extract from the plants col-

lected at monthly intervals contained a different quantity of nitrogen, a natural series was provided, in which each member had a different nitrogen content. Furthermore, this nitrogen is in that form in which it most probably occurs under natural conditions, in the plant itself.

Great care was taken to make up each extract in exactly the same concentration, 25 mgm. of extract per liter of sea water. This insured that any individual sample was directly comparable with all the others in the series. Oxygen determinations were made after one day of storage. The results are given in Table III along with the percentage

TABLE II

Effect of Added Nitrate on Decomposition of Chondrus Extract

Extract added (mg. per bottle)	Nitrate nitrogen (mg. per bottle)	Oxygen consumed (ml. per liter)		No. bacteria per ml.
		3 days	7 days	3 days
0	0	0.53	0.74	—
0	0.014	0.55	0.80	90,000
0	0.042	0.55	1.24	—
5	0	1.32	4.07	560,000
5	0.014	3.27	5.02	980,000
5	0.042	4.28	5.28*	—
5	0.140	5.28*	5.28*	3,200,000

* All oxygen consumed.

In this experiment the sea water was enriched by the addition of 1 mg. K_2HPO_4 per liter.

of nitrogen in each sample. It is readily seen that a correlation exists between the two and is most direct where nitrogen is low. This seems, therefore, to furnish definite evidence of the limiting effect of nitrogen on the decomposition of, at least one type of, organic material such as occurs in the sea. It suggests also that the low level of nitrogen during the summer may be partially responsible for the slow rate of decomposition.

While in the present discussion it is assumed that the decomposition is brought about by bacteria, the possibility that the oxygen is consumed by other organisms is not excluded. The water used may, undoubtedly, have contained larger organisms. It was surface water collected in Vineyard Sound and filtered through a No. 25 net of bolting silk. The bacterial considerations cannot be discussed here although evidence was obtained for the bacterial nature of the decomposition. However, the important fact remains that the consumption of oxygen on storing the

polysaccharide extract of *Chondrus* in sea water is correlated with the amount of nitrogen which it contains naturally.

TABLE III

Oxygen Consumption of Various Extracts of Chondrus crispus *

Extract	Nitrogen (per cent dry weight)	Oxygen consumption occasioned in one day by addition of extract (ml. per liter)
January	1.92	0.92
February	2.30	1.05
March	2.40	1.21
April	2.18	1.12
May	1.40	1.09
June	0.82	—
July	0.34	0.23
August	0.37	0.27
September	0.32	0.20
October	0.39	0.24
November	1.28	0.65
November	0.80	0.51
January	2.34	0.80

* The sea water used in this experiment showed an oxygen consumption of 0.55 ml. per liter in one day.

SUMMARY

The decomposition of *Chondrus crispus*, as measured by oxygen consumption, has been shown to be more readily accomplished on storing in sea water than that of its polysaccharide extract. This has been attributed to the higher nitrogen content of the former.

Inorganic nitrogen added to the polysaccharide extract of *Chondrus* increased its decomposition.

Samples of the extract containing different quantities of nitrogen have been found to decompose in direct proportion to the amount of nitrogen which they contain.

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THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON MICROBRACON HEBETOR SAY (HYMENOPTERA: BRACONIDAE) AND ITS HOST, EPHESTIA KÜHNIELLA ZELLER (LEPIDOPTERA: PYRALIDAE)

III. Effect of the Sting of the Parasite and of Two Chemical Agents on the Respiratory Rate and Quotient of the Host Larvæ (E. kühniella Zeller)

NELLIE M. PAYNE

Many species of parasitic Hymenoptera paralyze their hosts by stinging them, after which they lay eggs in or near the host. Although the gross effect of the sting on the host has long been known, yet the precise effect has been little studied. In this paper the change in oxygen consumption of the host larva after being stung by the parasite is measured and these results compared with those produced by ether and by orthodichlorbenzene.

Methods and Materials

The respiratory exchange of full-grown larvæ of *Ephestia kühniella* Zeller was measured with the manometer described by Krogh (1914) and modified by Bodine and Orr (1925). Baskets of copper gauze lined with cotton were hung on the glass hook which extended from the open end of the manometer. Larvæ placed in them were unable to move about but may have developed some muscular tension by struggling against the cages. Since the larvæ tended to be sluggish when undisturbed, they probably developed but little tension. Manometers were calibrated by weighing the mercury required to fill them. They were run for periods of two and one-half hours.

Larvæ to be paralyzed were exposed to adult females of *Microbracon hebetor* Say which pierce the host larvæ with their ovipositors, then lay eggs on or near them. Since the parasite does not begin to feed on the host until these eggs hatch, the host larvæ suffer no loss of material from the parasitic attack. Occasionally a host larva will writhe and struggle when the wasp is stinging it and for some time afterwards. Stung larvæ were placed in the manometer only when they were quiet.

Larvæ were also paralyzed by ether and by orthodichlorbenzene. A dosage of the reagent that would paralyze one-half the larvæ used and from which recovery took place in at least twelve hours was chosen. Since there were wide differences between the susceptibility to chemical agents even among larvæ hatched from the eggs of the same female, considerable experimentation was required to find a suitable dose. Larvæ were given sufficient anesthetic to paralyze but not to kill them. Such an amount may be designated as the median-paralytic dose. Larvæ to be paralyzed by a chemical agent were placed in lots of twenty in a cylindrical wire cage made of screening. These cages were fastened to a hook blown onto a glass tube extending through the ground-glass stopper of a fumigation flask. The reagent was introduced into the flask through a glass tube which extended but a short distance below the stopper. Both this tube and the longer one on which the insects were hung were furnished with ground-glass stopcocks. The flasks were shaken from time to time.

After the median-paralytic dose had been established for both ether and orthodichlorbenzene, individual insects were transferred directly from the fumigating flasks into the manometers, where oxygen consumption was determined. The results obtained directly after the larvæ were treated are recorded under " Period I " in the tables. A second group of determinations, made six hours after treatment, is recorded under Period II. Larvæ removed from the flasks directly and those which had been in the manometer were kept in individual cages to see if they recovered. Only those measurements obtained from larvæ able to resume normal movement and feeding and finally to pupate appear in the tables. Thus respiration of paralyzed larvæ but not of dying ones was measured.

Oxygen consumption was measured at seven different temperatures namely 10° C., 15° C., 20° C., 25° C., 27° C., 30° C., and 35° C. Manometers were placed in water baths which controlled temperatures to $\pm$ 0.5° C.

Determinations were repeated until results from three larvæ, all of which recovered from the anesthetic, were obtained. Oxygen consumption of three individual larvæ paralyzed by parasitic sting was measured. Determinations on the oxygen consumption of three normal larvæ, untreated with anesthetics and not exposed to the attacks of the parasite were used as control. Carbon dioxide production was determined for each of the larvæ used and the respiratory quotients calculated. Results in the tables are averages of three determinations each. The temperature coefficient or Q_{10} was calculated between the intervals of temperature at which determinations were made.

Results

Oxygen consumption of normal untreated larvæ increased directly with the temperature until 32° C., at which point it decreased sharply. The respiratory quotient, however, showed little or no change until the temperature rose to 32° C. Between 30° C. and 32° C. the respiratory quotient dropped from 0.91 to 0.83 (see Table I). Since the full life cycle of the Mediterranean flour-moth cannot be completed at temperatures much above 32° C., it would appear that the lowering of the

Table I

Comparative data on oxygen consumption of normal full-grown Ephestia larvæ and Ephestia larvæ stung by Microbracon hebetor

Temperature in degrees Centigrade	Oxygen consumption in cc. per gram per hour		Q_{10}		Respiratory quotient	
	Normal larvæ	Stung larvæ	Normal larvæ	Stung larvæ	Normal larvæ	Stung larvæ
10	24.2	18.6			0.91	0.92
15	36.4	18.2	2.18	1.00	0.92	0.89
20	42.0	21.	1.73	1.12	0.89	0.91
25	53.6	25.3	*1.47 (2.54)	*1.32 (2.04)	0.92	0.88
27	61.5	27.5	†1.04	†1.72	0.93	0.93
30	72.3	31.	1.72	1.476	0.91	0.87
32	54.7	26.3			0.83	0.81

* Between 15° C. and 25° C. ( ) Between 20° C. and 25° C.
† Between 20° C. and 27° C.

respiratory quotient into a range indicating protein oxidation indicates some degree of injury.

Larvæ treated with ether were not only motionless but appeared soft and flaccid. The relaxation produced by the ether was apparently reflected by the lowered oxygen consumption of the etherized larvæ compared with the normal. As the larvæ recovered they used more oxygen. At all temperatures their oxygen consumption was less than that of untreated larvæ, but at low temperatures this difference was not as marked as at high. Etherized larvæ showed a similar decrease in oxygen consumption between 30° C. and 32° C. to that of untreated larvæ (see Table II). After six hours oxygen consumption had returned nearly to normal levels. After twenty-four hours etherized larvæ could not be distinguished from untreated by either their appearance or by their oxygen consumption. Etherized larvæ from which the records were made were able to spin cocoons and to emerge as normal adults.

Directly after treatment with orthodichlorbenzene, larvæ of *Ephestia* were tense and appeared to be in a state of rigor. They held their heads stiffly upright in a sphinx-like position from a tense motionless body. Even six hours after treatment the larvæ appeared somewhat stiffer than normal. In contrast to the larvæ treated with ether, those treated with orthodichlorbenzene showed a greater oxygen consumption than did normal larvæ, over the temperature range from 10° C. to 30° C. inclusive. (Compare Table I, Column 2 and Table II, Column 2.) At 32° C. the oxygen consumption of larvæ treated with orthodichlor-

TABLE II

Oxygen consumption of full-grown Ephestia larvæ treated with orthodichlorbenzene (1 mgm. per liter), and with ether (.568 gm. per liter)

Temperature in degrees Centigrade	Oxygen consumption in cc. per gm. per hr.				Q_{10}			
	Period I		Period II		Period I		Period II	
	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$	Larvæ exposed to ether	Larvæ exposed to $C_6H_4Cl_2$
10	22.3	24.3	23.4	23.2				
15	33.5	39.3	35.7	37.1	2.99	2.12	3.04	3.2
20	37.4	47.1	44.1	43.5	1.677	1.98	1.84	1.87
25	42.7	58.5	52.3	52.7	*1.304	*1.49	†1.46	1.42
					(2.28)	(2.48)	(2.39)	(2.46)
27	45.6	67.4	61.	60.9	1.95	†2.04	†1.51	2.0
30	52.1	80.5	69.9	73.1	1.39	1.73	1.58	1.68
32	45.8	46.7	51.6	53.8				

* Between 15° C. and 25° C. ( ) Between 20° C. and 25° C.
† Between 20° C. and 27° C.

benzene was less than that of the untreated. This greater decrease in oxygen consumption may indicate that larvæ exposed to orthodichlorbenzene are more susceptible to injury by high temperatures than are normal larvæ.

Since larvæ exposed to fatal doses of orthodichlorbenzene showed a gradual decrease in oxygen consumption during the period they were in the manometer, they could be distinguished from larvæ exposed to a non-fatal dose. A marked decrease in oxygen consumption during the time that larvæ were in the manometer was interpreted as a measurement of a death process rather than of the specific effect of orthodichlorbenzene.

The respiratory quotient of larvæ treated with orthodichlorbenzene did not differ significantly from that of normal larvæ. In both cases the

respiratory quotient dropped from the carbohydrate to the protein range at 32° C. Both normal larvæ and those treated with orthodichlorbenzene showed a marked decrease in oxygen consumption at the same point as that at which the respiratory quotient decreased.

Larvæ stung by *Microbracon* closely resembled those treated with ether. In both cases the larvæ were soft, relaxed and motionless. They consumed less oxygen than either normal larvæ or etherized larvæ throughout the range of temperature used. As measured by oxygen consumption, the sensitivity to temperature change of stung larvæ was far less than for normal larvæ. Thus between 10° C. and 15° C. there was no increase in oxygen consumption by the stung larvæ. At 30° C. the oxygen consumption of the stung larvæ was about that of normal larvæ at 15° C. (see Table I). At 32° C. the oxygen consumption of the stung larvæ decreased from what it had been at 30° C. Also at 32° C. the respiratory quotient showed a marked decrease.

The level of oxygen consumption of the stung larvæ remained approximately constant for at least a week after they were stung since measurements taken a week after their exposure to *Microbracon* were nearly the same as those taken directly after their paralysis. The magnitude of change produced by the *Microbracon* was far greater than that caused by either of the chemical agents used. In the experiments reported here, both ether and orthodichlorbenzene produced a reversible change while the sting of *Microbracon* produced an irreversible change. If the dosage of ether or of orthodichlorbenzene be increased to the point of irreversible change the larvæ die rather than remain quiescent as they do when stung by *Microbracon.*

A few experiments were tried to see if either orthodichlorbenzene or ether would affect larvæ stung by *Microbracon.* As far as could be detected from measurements on respiratory rate and quotient neither orthodichlorbenzene nor ether had any effect on larvæ stung by *Microbracon.* Furthermore, the stung larvæ treated with orthodichlorbenzene did not stiffen nor those treated with ether become more flaccid. Apparently the *Microbracon* sting had already destroyed or damaged the mechanism on which these drugs could act.

Discussion

Levels of respiratory metabolism of *Ephestia* larvæ stung by *Microbracon* are so low and so insensitive to temperature change as to suggest those of cells in diapause such as the "blocked" embryonic grasshopper cells reported by Bodine (1934). The resting condition of the *Ephestia* larvæ differed, however, from that of the embryonic grasshopper cells in

that the rest of the grasshopper embryos could be broken with greater ease than could that of the larvæ stung by *Microbracon*. The author has never observed an Ephestia larva recover from *Microbracon* sting. Mr. H. C. Donohoe, in an unpublished communication, informed the author that he had seen *Ephestia* larvæ recover from the sting of the parasite but that recovery was very rare.

Although temperature rise from 10° C. to 15° C. did not change the respiratory rate of *Ephestia* larvæ stung by *Microbracon* and temperature change from 15° C. to 30° C. increased the rate in stung larvæ only one-third as compared to twice in normal larvæ, still temperature rise from 30° C. to 32° C. was associated with a drop in respiratory rate for the larvæ stung by *Microbracon*, as it was so associated with normal larvæ. Thus the stung larvæ, while insensitive to changes in temperature through the range through which normal development occurs, are susceptible to injury from high temperature.

Throughout the temperature range used in the experiments with *Ephestia* larvæ, the respiratory quotient of the larvæ stung by *Microbracon* did not differ from that of normal larvæ. Both were in the carbohydrate range from 10° C. to 30° C.; both dropped to the protein range at 32° C. In so far as the respiratory quotient indicates the type of metabolism, a lack of difference between the respiratory quotient of stung larvæ and of normal larvæ would indicate the same type of metabolism in both. Differences not measured by the respiratory quotient might well occur.

Neither the effect of ether nor that of orthodichlorbenzene duplicated that of the *Microbracon* sting. Ether did produce flaccidity in the larvæ and did lower the respiratory rate. It was impossible to obtain as low a rate with ether as the *Microbracon* sting produces, without killing the larvæ. Orthodichlorbenzene produced a rigidity in the larvæ and also increased respiratory metabolism. Neither chemical agent affected the respiratory quotient.

Hartzell (1935) found that lesions produced by the killer wasp, *Specius speciosus* Dru., in the nervous system of the cicada, *Tibicen pruinosa* Say, were similar to those found in insects killed with triorthocresyl phosphate and the pyrethrins. Injections of formic acid and of acetic acid in meal worms (*Tenebrio molitor* L.), however, failed to produce the lesions characteristic of paralysis.

The finding of Hartzell (1935) of lesions sufficient to be demonstrated microscopically in the nervous system of a cicada stung by a wasp may be correlated with the profound differences in the muscular tonus between normal larvæ and stung larvæ. In turn these alterations

of the nervous system associated with marked changes in respiratory rate may point to an intimate connection of the nervous system with respiration.

Summary

1. Larvæ of *Ephestia kühniella* Zeller, when stung by the parasite *Microbracon hebetor* Say, become soft, flaccid and motionless.

2. Associated with the flaccidity of the stung larvae is a greatly lowered respiratory rate through the temperature range used, namely 10° C. to 32° C.

3. Not only does the *Microbracon* sting lower the respiratory rate but it decreases the sensitivity of *Ephestia* larvæ to change in temperature to such an extent that between all temperature intervals used the Q_{10} of larvæ stung by *Microbracon* was less than that of normal larvæ. Between 10° C. and 15° C., the Q_{10} was 1.

4. Orthodichlorbenzene in a median paralytic dosage (just sufficient to cause a reversible paralysis in half the larvæ) produced stiffness and rigidity in *Ephestia* larvæ. Associated with this stiffness was a marked increase in respiration rate beyond that of untreated larvæ except at 32° C. where it fell below that of normal larvæ.

5. Ether in a median paralytic dosage produced flaccidity in *Ephestia* larvæ. Associated with this flaccidity was a marked decrease in respiratory rate, but at no temperature was the decrease produced by ether of the same magnitude as that produced by the *Microbracon* sting.

6. Unlike the respiratory rate, the respiratory quotient was not affected by the parasite sting nor by either of the two chemical agents used.

7. Rise in temperature to the point at which the rate of development of *Ephestia* decreases, namely 32° C., was associated with a drop in respiratory rate and a decrease in respiratory quotient from the range indicating carbohydrate metabolism to that indicating protein. Not only normal larvæ, but also those treated with ether or orthodichlorbenzene or stung by the parasite, showed a decrease both in rate and quotient at 32° C.

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AUTOTOMY IN THE BRACHYURAN, UCA PUGNAX

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A study of the extent to which the anomuran *Porcellana platycheles* will autotomize its legs on single stimulation of successive appendages has shown (Hoadley, 1934) that there is a clear-cut and fundamental difference between the behavior of males and of females bearing eggs. This form is ideal for such experiments in that gently seizing the leg between the points of blunt forceps will ordinarily be followed by autotomy. Males tested in the above fashion autotomized an average of 5.57 + legs per animal (69.6 per cent), the distribution of the autotomies being 4 for the first four trials and 1.57 + for the second four. Quite in contrast to this, the gravid females cast an average of two legs in the eight trials (25 per cent), the distribution being 1.5 and 0.5 in the first and second four trials respectively. Unfortunately but one female not bearing eggs was available at the time of the examination of this material, but a test of that animal showed five legs autotomized (62.5 per cent) (distribution 4 and 1), a figure which compares more favorably with the condition encountered in the male than with that of the remaining females. The suggestion was made, therefore, that the functional state of the animal bearing eggs was different from that of the male and that of the non-gravid female and that this difference was directly reflected in the extent of the autotomization. The present report is based on an examination of autotomy by males, egg-bearing females, and females not carrying eggs, in the brachyuran *Uca pugnax*.

The occurrence of autotomy in the decapod Crustacea and the methods by which this is accomplished have been examined in detail by Wood and Wood (1932). That of the fiddler crab, *Uca pugnax*, has been found by them to be a true autotomy in contrast to autospasy and autotilly, which may be of great importance in other forms. When the autotomy of *Uca* is compared with that of *Porcellana* described above, however, it is at once evident that while *Porcellana* casts its legs on the slightest provocation and hence care in handling the animal is essential, *Uca* casts its legs only after injury so that a relatively harsh treatment is necessary to induce the reflex.

The animals used in the following experiments were obtained from

the shore of Rhode Island [1] and brought immediately to the laboratory where the experiments were performed. Two lots of animals were worked with, one obtained in August of 1934 when the females were not bearing eggs and the other in June of 1935 when over half of the females were carrying eggs attached to the abdomen. All of the animals used in the experiments were mature. It was necessary in some of the experiments to use a few animals which had already cast one of the legs but in no case was an animal with more than one leg lacking employed.

Autotomy was induced by injuries of two sorts. In the first a limb was grasped and crushed between the jaws of a pair of plier forceps with a quick motion in order that the leg should actually be held as short a time as possible. Such injuries were generally made in the carpus though the exact position on the segment varied slightly and in several instances the injury was in the distal portion of the meros. It was soon found that holding the animal either by the body or by the leg tested facilitated the autotomy of the member so that the animal was not held during the infliction of the injury. That fact, together with the effort to accomplish the injury in as short a time as possible, led to slight variations in the actual extent of the trauma. In the male, the large claw of the cheliped was crushed across the middle at the base of the dactylus and consequently the injury there was relatively great.

The second type of injury inflicted to induce autotomy was cutting. Legs were cut with a heavy pair of scissors at or near the joint between meros and carpus, removing the carpus and propus. The claw of the cheliped of the male had to be cut by means of a small pair of bone forceps, the cut being located in the same region as the injury by crushing mentioned above. Both types of experiment were carried out in clean moist crystallizing dishes; in addition, crushing experiments were performed in crystallizing dishes, the bottoms of which were covered by filter paper. Only females bearing eggs and males were used in the cutting experiments.

The results of the experiments have been summarized in Table I. The letters following the year indicate the order in which the experiments for that year were done. While every effort was made to perform the experiments in the same way, it is but natural that those done in the same year should be most similar and that those done successively in any one year should be most nearly alike in execution. Thus, of the experiments in which appendages were crushed on glass, 34A (♂'s) and 34B (♀'s) are comparable, as are also 35A (♀'s with eggs) and 35B (♂'s) on the one hand, and 35G (♀'s with eggs), 35H (♀'s without

[1] I wish to thank Dr. F. C. Chace for the *Uca* which he was so kind as to collect for me.

eggs) and 35I (♂'s) on the other. It is evident when these are compared that the stimulation in 35A and 35B was not as effective as in 35G, 35H and 35I. This will be dealt with in some detail below. The results themselves seem to be comparable, however, for when the number of legs autotomized by the males is compared with the number autotomized by the female bearing eggs it is found to be very similar in the two cases

TABLE I

Method	Sex	First 5 trials	Second 5 trials	Total	First 5 trials / Second 5 trials	Year and series	Number of animals	Average first 5 trials	Average second 5 trials	Average total		
Crushing on glass	♂	41	23	64	1.81−	34A	9	4.56−	2.56−	7.1+	♂/♀ = 1.22+	Averages— ♂'s = 7.1 ♀'s = 6.65 ♀ eggs = 4.4
	♀	37	21	58	1.76+	34B	10	3.7	2.1	5.8		
	♀ with eggs	15	5	20	3.0	35A	5	3	1	4.0	♂/♀ eggs = 1.60	
	♂	19	13	32	1.46	35B	5	3.8	2.6	6.4		
	♀ with eggs	16	8	24	2.0	35G	5	3.2	1.6	4.8	♂/♀ = 1.04 ♂/♀ eggs = 1.62	
	♀	16	14	30	1.14+	35H	4	4.0	3.5	7.5		
	♂	23	16	39	1.44−	35I	5	4.6	3.2	7.8		
Crushing on filter paper	♂	21	15	36	1.40	34C	5	4.2	3.0	7.2	♂/♀ = 0.94	
	♀	20	17	37	1.18−	34D	5	4.0	3.4	7.4		
	♀ with eggs	15	9	24	1.67	35E	5	3.0	1.8	4.8	♂/♀ = 1.10† N.B. anterior appendages first in every case	
	♂	17	11	38	1.54+	35F*	5	3.4	2.2	5.6		
Cutting on glass	♂	11	5	16	2.2	34E	5	2.2	1.0	3.2		
	♀ with eggs	11	1	12	11.0	35C	5	2.2	.2	2.4	♂/♀ eggs = 1.5	
	♂	12	6	18	2.0	35D	5	2.4	1.2	3.6		

Average ♂/♀ = 1.067− ♂/♀ Eggs = 1.57+

* The large claw was injured first in each of the experiments of this series; see discussion in text.

† Omitted in the average.

being expressed by the ratios 1.60:1 and 1.62:1 respectively. When the average total number of legs autotomized by the three classes of animals is examined, it is found that, with one exception, the largest number is cast by the males; the females without eggs come next; the females with eggs cast the smallest number. It will also be noted that the females without eggs cast a relatively smaller number of legs in August

than they did in June. Compared with the males, the index for such animals is 1.22 in August as compared with 1.04 in June, the behavior at the latter time being similar to that of the males. It appears quite evident from the tabulated data that the female bearing eggs behaves quite differently in autotomizing its legs from the male and from the female not bearing eggs. It is also evident that the greater difference is to be found in the second five trials. This can be appreciated most easily by examination of the column in which the relation between the number of legs lost in the first five trials is compared with the number lost in the second. If the number lost in each series is approximately the same the ratio will approach 1 while if the number diminishes materially it will rise. In all of the first group the higher quotients are to be found in animals which are females and carry eggs.

The second type of experiment differed from the first only in that the bottom of the dish in which the animals were placed during the experiment was covered with filter paper. Examination of the totals would appear to indicate that the behavior in this case is similar to that in the preceding. The exception is found in the case of the males of 35F which showed an average total of 5.6 which is well below that of the previous series and also well below those of the males and of the females without eggs used in 34C and 34D. Reference to the protocol of the experiment shows, however, that in this series (35F) the large claw was the first to be injured and this has evidently had a marked effect on the subsequent behavior of the individuals. This might be explained either on the basis of the extent of injury or on the basis of some aid in autotomy rendered by the large claw. While the large claw is used directly to rid the animal of the injured member at times, this is by no means usual and could not, I believe, account for the discrepancy in the results. It is also of interest to note that when the ratio between the autotomies in the first five trials and the second five is examined in this entire group it is found that the number of autotomies is relatively high in the second five and that hence the index is lowered. Again, this is not true of the males in 35F. While no quantitative data are available on this point it should be mentioned that on the filter paper, the amount of bleeding which takes place is greater than when the animal is in the clean glass dish. The possible significance of this will be discussed below, more particularly in relation to the cutting experiments. As in the previous group of experiments, the relation between the total number of autotomies by males and by females not carrying eggs is approximately the same.

Only three sets of experiments were made cutting the appendages of the animals upon glass. Two of these were done in 1935 and one in

1934. Comparing the males of 34E with those of 35D it is found that in both cases the number of legs autotomized is very low, being (as averages) 3.2 in the first instance and 3.6 in the second. These figures are very similar and are characteristic of the individuals of each series of experiments. The females bearing eggs showed a similar low average of 2.4. The immediate result of the experimental procedure differs from that recorded above in that there is far more bleeding after cutting than after the crushing previously discussed. When the males of the 1935 series are compared with the females it is found that the relation approaches that of the crushing experiments, being 1.5. When the number of autotomies in the first five trials is compared with the number in the second five, it is found that for the males the quotient is relatively high, being 2.2 and 2.0; but this is low compared with that for the females bearing eggs, which is 11.0, the highest encountered in any of the experiments. The relation between the males of the two years is 1.11, which is close agreement.

The data obtained in the experiments may be dealt with in two ways. It is possible to compare the total number of legs cast by each group according to the animals composing it, or the number of legs cast in the first five trials may be compared with the number cast in the second five and the quotients examined. The results of each method yield information which is interesting and hence will be considered separately.

The relation between the total number of legs autotomized by comparable groups of animals will be considered first. The most casual examination of the table will show that the males and the females without eggs consistently cast more appendages than did the gravid females. The difference is so great that when the males are compared with females bearing eggs which were tested at the same time the ratio is as 1.57 + (average) is to 1. For the reasons stated in the table the results of tests 35E and 35F have been omitted from this average. It is of especial interest to note that while the method employed may have a great effect on the totals obtained it has not modified the relation between the totals (see " cutting on glass ").

When the autotomies of females not bearing eggs are compared with those of the males it is found that in the 1935 tests (June) the results for the two sexes are essentially the same. In the tests of the 1934 series it might at first be thought that the averages in 34A and 34B showed a difference in behavior. A comparison with the results of 34C and 34D which are also comparable leads to the conclusion that the discrepancy in the former case is the result of unequal stimulation rather than of a difference in the animals. This is not unlikely for 34A and 34B were the first *Uca* to be tested. When the results obtained in all

of the tests are averaged it is found that the relation between autotomy by the male and by the non-gravid female is as 1.067 — is to 1, the difference not being significant. The variation in results between more widely separated tests of the same year and between tests of different years is to be explained on the basis of manipulation.

A comparison of the number of autotomies in the first five trials with the number in the second five should prove most interesting and should yield important information, for by this method groups of stimulations of individuals are dealt with and these represent tests which are most similar in every respect. Several general statements may be made based on the results of this comparison. It may be stated definitely that in comparable experiments the relative number of autotomies in the first five trials in females bearing eggs is consistently higher than in the remaining females or in the males. On the other hand, the differences encountered in this ratio between animals of this second class (34A and B; 35B, H, and I) of the first group alone is fairly large as is the difference between the gravid females (35A and G). While both the latter values are above those of the first class, the difference between the highest value of the first class and the lower value of the second is relatively small. The ratio in itself becomes more significant after the two components are examined. Insofar as the first five trials are concerned the results are much the same whether the crushing be on filter paper or on glass; as for the second five, the average on the filter paper is in all cases high. This would lower the value. The reverse is particularly marked in the cutting experiments where in males the average of the first five trials is approximately half that in the crushing experiments while that of the second five is nearer one-third. The females with eggs behaved much as the males in the first five trials but in the second five autotomy was practically suppressed so that the relation between the two rises to a value of 11.0. In this connection it should be stated that the results of a few experiments on cutting on filter paper indicate that in that case also there is a similar reduction in the number of autotomies in males. In 35F we find that both the first and the second set have been reduced, the second more than the first. This results in a value for first/second of 1.54 + which approaches that of the female bearing eggs as did the total number of autotomies (5.6 av.).

It has already been mentioned above that relatively harsh treatment is necessary to induce autotomy in *Uca*. Sensory receptors associated with hairs are present in the cuticle covering the limbs. The tactile stimulation of the appendage of *Porcellana* is sufficient to induce the casting of a leg but in *Uca* the actual injury of the member is essential. This, in turn, must result in much more violent stimulation of afferent

sensory nerves. As may be seen in the data presented above, the degree of stimulation in different experiments differs and with this variation there is an accompanying variation in the number of autotomies. Evidence of the same sort may be derived from the comparison of cutting experiments and crushing experiments. In the former the number of autotomies is far lower than in the latter. Similarly it might be expected that the extent of stimulation both in degree and in duration would be greater in the case of crushing than in the case of cutting. This probably does not account completely for the discrepancy in the two cases as the amount of bleeding observed differs.

The blood of the Crustacea coagulates very readily. Ordinarily there is relatively little loss of blood following traumatic injury to the limbs. It has been stated above that there is a greater loss of fluid when the appendage is crushed on filter paper than when crushed on moist glass. The loss is far greater when the appendage is cut at or near the base of the carpus. With successive stimulation the latter type of injury eventually results in the loss of considerably more blood than does the crushing. This may contribute to the difference in the behavior of the two types of material.[2] Similarly the extent of the injury to the large claw on crushing must result in the loss of more of the body fluid than would injury to other appendages. This may in part account for the reduction in the total number of autotomies in series 35F. The amount of bleeding following autotomy, i.e. at the breaking joint, is relatively small so that the act in itself tended to restrict the loss of body fluid by the injured animals in the majority of the crushing experiments.

The autotomy reflex of the Crustacea as generally conceived involves the transmission of stimuli along the sensory neurones and transmission of motor impulses by the motor neurone to at least the autotomizing muscle. The path is presumably completed centrally in the ventral ganglion. There are very few motor nerve fibers in the segmental nerves of the crab, indicating that whole muscles and possibly even different muscles may be innervated by collaterals of a single neurone. In the crab the fifth to the thirteenth lateral nerves all have their origin in a common ventral ganglionic mass, the ninth to the thirteenth innervating the five appendages as well as the muscles of the body wall of these segments. In the experiments reported here as well as those on *Porcellana* referred to above, the reflexes which are activated by successive stimulations have a cumulative effect and lead to an inhibition and eventual failure of the response even when the stimulations involve re-

[2] Dr. John H. Welsh informs me that some collectors bleed crabs in order to prevent their casting appendages when put into preservatives. He himself has used this method successfully.

ceptors situated in different appendages. In the male of *Porcellana* the inhibition first became evident after the fifth successive appendage was stimulated; in the male of *Uca* the inhibition is usually noted after the fourth stimulation. In females bearing eggs, inhibition appears between the first and the second trials in *Porcellana,* and after the third in *Uca.* Females not bearing eggs appear to behave as males in *Uca* and the indications are that the same is true for *Porcellana.* That there is actually an inhibition and that it is gradually built up is demonstrated by the fact that without exception the number of autotomies in the first five trials is greatly in excess of those in the second five. It is also apparent that the inhibition becomes effective more rapidly in the female bearing eggs than in the male for the relation between the number of legs cast in the first five trials and in the second five yields a higher quotient in the first case. These facts suggest strongly the formation of a block most easily explained in terms of a chemical inhibitor. This inhibition may be effected in the periferal mechanism or in the fused central ganglionic region. The fact that bleeding tends to reduce the number of autotomies also leads us to the conclusion that the inhibitor is of a chemical nature and that it is readily eliminated through the blood, accumulating more rapidly when the amount of blood in the body is reduced.

One additional observation deserves mention in that it, too, suggests a mutual effect, though in this instance the effect is in terms of stimulation rather than of inhibition. In several instances a delayed autotomy has been observed which took place on the stimulation of another appendage. This is well illustrated by a case in which the third leg stimulated failed to be cast at the time but gave a second response which was successful when the fifth successive leg was stimulated. Both reflexes were typical and the leg was eventually autotomized in the usual fashion. Apparently the nerve impulses in the neighboring units were responsible for the autotomy, possibly through the production of diffusable humoral agents which, in turn, effect the activation of previously stimulated components.

In conclusion it should be emphasized that the evidence for the existence of inhibiting and stimulating substances is indirect and that they have not been demonstrated. However, the behavior of both *Porcellana* and *Uca* may be most readily understood on some such basis. The method of performing the experiment has a definite influence on the kind of result obtained. Not only should a study be made of the components of the blood, but, recognizing the presence of the fused ventral central ganglia, similar experiments should be performed on the crayfish or some like form in which the ganglionic enlargements from which the

walking legs are innervated are segmentally arranged and discrete, and where, therefore, mutual influence in the central mechanism would be less likely. The experiments do demonstrate very clearly that, in respect to the extent to which legs are autotomized, there is a fundamental difference between females bearing eggs, on the one hand, and females without eggs and males on the other.

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POLAR BODY EXTRUSION AND CLEAVAGE IN ARTIFICIALLY ACTIVATED EGGS OF URECHIS CAUPO

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Introduction

In recent articles, Hiraiwa and Kawamura (1935, 1936) report some parthenogenesis experiments on eggs of *Urechis unicinctus*. As their results differ in some respects from those of Tyler (1931*a*, *b*, 1932*a*, *b*) on *Urechis caupo* and as one of us (B.) intended to continue the unfinished studies of Bělař on the cytology of parthenogenesis, we decided to test the methods of the Japanese authors on the Californian *Urechis*. The principal point of the mentioned difference is that, in the experiments of Hiraiwa and Kawamura, high percentages of cleavage were obtained in batches of eggs most of which had extruded polar bodies, whereas, in the experiments of Tyler, eggs that extruded polar bodies almost invariably failed to divide. Hiraiwa and Kawamura used heat, hypo- and hypertonic sea water, KCN sea water, and, most successfully, ammoniacal sea water, for which method alone they present the data on polar body extrusion and cleavage. Tyler had used only anisotonic sea water.[2]

It was considered advisable to isolate the various types of eggs according to their behavior in the maturation divisions, a precaution which Hiraiwa and Kawamura failed to take. This becomes of especial importance in view of the fact that after certain treatments and at certain stages blisters may appear on the surface of the egg that may easily be mistaken for polar bodies. The Japanese authors do not mention these blisters though they show them in their drawings. Also it is possible, as was pointed out by Hiraiwa and Kawamura, that when two polar bodies are present on the egg, they may represent the first and second

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[2] The Japanese authors erroneously attribute to Tyler the view that cleavage cannot occur in eggs which have extruded both polar bodies. Although it was found that such eggs failed to divide, the possibility was admitted that "by means of other agents or by additional treatment the eggs that extrude two polar bodies may be made to develop" (Tyler, 1931*a*, p. 209).

polar bodies or the divided first polar body or two "second" polar bodies. Isolation of the eggs helps to decide these points.

It may be stated at the outset that the conclusion of Hiraiwa and Kawamura could be confirmed that, after certain treatments with ammoniacal sea water, eggs divide after polar body formation. However, certain reservations must be made, since only low percentages of cleavage were obtained, and also the same exposure that gives cleavage generally produces abnormalities in the maturation divisions, so that it is not certain that the divided eggs with two polar bodies are haploid. This and related points will be cleared up in the cytological investigation. Where one or both maturation divisions are submerged, we find that cleavage almost invariably follows and the type of cleavage is related to the polar bodies produced.

Hiraiwa and Kawamura also report that, by means of a second treatment with ammoniacal sea water applied after the extrusion of the second polar body, eggs which ordinarily would not do so, may be made to divide. Repetition of this experiment on *Urechis caupo* failed to confirm the result. They report too that the "poorly activated" eggs described by Tyler did not appear in their experiments. Using the ammoniacal sea water treatment with *U. caupo*, however, we find again this type of egg after certain treatments, and its behavior is the same as in the hypotonic sea water experiments.

A new agent was also tried in the experiments reported here, namely ammoniacal dilute sea water which gave high percentages of cleavage, without polar body formation. The kind of cleavage, however, differs in an interesting manner from that obtained by the ammoniacal sea water treatment. Acidified sea water was also used in some experiments with results that were essentially the same as in the hypotonic sea-water experiments.

Treatment with Ammoniacal Sea Water

Urechis eggs may be activated by sea water containing ammonia in a wide range of concentrations. Solutions ranging from 0.02 molar to 0.002 molar NH_3 in sea water were employed. The minimum exposure necessary to obtain 100 per cent activation is, of course, shorter the more concentrated the solution. A typical run is presented in Table I. The solution employed in this experiment is the same as that used in some of the experiments of Hiraiwa and Kawamura, and the activating exposures are roughly the same as in their experiments. At other concentrations the results with *Urechis caupo* also approximate those with *U. unicinctus* when allowance is made for the temperature differences.

Also the exposures giving cleavage are in part similar in both cases. But certain differences are evident. In the *U. unicinctus* experiments no cleavage was obtained after short exposures to the activating solution, whereas we obtain cleavage after exposures too short to activate all of the eggs. The eggs that divide after the short treatment are all of the "poorly activated" type previously described and figured (Tyler, 1931*a*, Figs. 27 to 30). They produce no polar bodies. Hiraiwa and Kawamura state that they could not find any such eggs in *Urechis unicinctus*. After somewhat longer exposures, ranging in the tabulated case from 2 to 7 minutes, 100 per cent activation is obtained but none

TABLE I

Activation with ammoniacal sea water. Solution = 0.01n NH_3 in sea water, temperature = 20° C.

Length of exposure	Total activation	Poorly activated	Polar bodies			First cleavage		
			0	1	2 or 3	2-cell	3-cell	4-cell
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
¼	40	70	75	5	20	60	3	2
½	90	30	—	—	—	20	0	0
1	100	15	15	0	85	15	1	0
2, 3, 5, and 7	100	0	0	0	100	0	0	0
10	100	0	10	20	70	20	10	2
15	100	0	40	30	30	10	40	45
20	100	0	—	—	—	10	45	25
25	100	0	60	15	25	5	20	15
30	100	0	—	—	—	5	10	10
40	100	0	100	0	0	0	0	0

of the eggs divide. This confirms roughly the cleavage-activation relation obtained with hypotonic sea water (Tyler, 1931*b*).[3] But upon still longer exposures cleavage is obtained, rising to about 90 per cent, and then dropping again to zero while the activation remains 100 per cent. Data on the number of polar bodies extruded are also given in Table I. It is at once apparent that after 2 to 7 minutes exposure, which gives 100 per cent activation but no cleavage, both polar bodies are extruded by all the eggs. After the longer exposures giving increasing percentages of cleavage, the proportion of eggs that extrude two polar bodies decreases. From the figures in the table it would appear that at these exposure times eggs with two polar bodies may divide. However, it must be pointed out that these counts are only approximate, and also that it is especially after such exposures that blisters very much re-

[3] See also Dalcq, Pasteels and Brachet (1936).

sembling polar bodies may appear. In Fig. 3 a blistered egg is shown and in Fig. 4 the same egg, ten minutes later, shows the blisters gone and no polar bodies present. Some eggs become covered with blisters so that polar bodies, if present, are completely obscured. The blistering usually appears at some time between extrusion of the second polar body and cleavage, and often reappears during cleavage. In many cases the blisters might easily be mistaken for polar bodies. Preserved eggs would, of course, be better material on which to make the counts, but the isolation experiments reported below give a satisfactory basis for the conclusions.

Cleavage of Eggs Isolated According to Number of Polar Bodies Produced

The eggs that divide after short exposures to the ammoniacal sea water all fail to extrude polar bodies just as in the hypotonic sea water experiments. One hundred such eggs were isolated from a batch

Table II

Polar body extrusion and cleavage. Eggs treated for 15 minutes with 0.01 n NH_3 in sea water. Isolated 60 to 80 minutes later. Eighty-five to 90 per cent cleavage in main batch.

	Number of eggs	Number cleaved
No polar bodies	47	47
One polar body	12	12
Two polar bodies	74	43

treated for 1½ minutes with 0.005 n NH_3 in sea water. All of the eggs divided into two cells at 2 to 2½ hours after treatment. No signs of submerged maturation spindles occurring were noted in the living egg, and it is likely that the first cleavage division is the equivalent of the first maturation division as in the case of eggs "suboptimally" activated with hypotonic sea water (Tyler, 1932*a*).

The eggs that divide after the longer exposure behave differently. The first cleavage may give two, three or four cells. There are two types of three-cell cleavage. One type produces three approximately equal-sized cells directly (Fig. 10). The other first attempts to go into four cells, then the cleavage plane between two of the cells disappears giving a three-cell egg in which one of the cells is equal in size to both of the others (Fig. 14). This type should be considered a four-cell cleavage. There are also two types of two-cell cleavage, one of which

is derived from an attempted three-cell cleavage. The different types of cleavage correspond to differences in polar body extrusion as will be shown below.

In order to determine the relation between polar body extrusion and cleavage, eggs that were given a treatment with ammoniacal sea water sufficient to induce a high percentage of cleavage were isolated according to whether they produced two, one or no polar bodies. Table II contains data of this sort from four experiments. The eggs were isolated at about 20 to 40 minutes after the time of appearance of the second polar body. It may be seen from the table that all of the eggs with no polar bodies and with one polar body divide and also more than half

TABLE III

Polar body extrusion and cleavage. Eggs treated for 15 minutes with 0.01n NH_3 in sea water. Isolated immediately after appearance of first polar body and again at time of appearance of second polar body. Seventy-five to 90 per cent cleavage n main batch.

	Polar bodies produced	Number of eggs	Cleavage at 1½ to 2½ hours			Cleavage at 3½ to 5 hours			
			2-cells	3-cells	4-cells	2-cells	3-cells	4-cells	>4-cells
From eggs with no polar bodies	None	39	4	10	24	0	0	0	38
	1	9	2	6	1	0	1	0	8
	2	12	11	1	0	0	0	1	11
From eggs with one polar body	1	2	1	1	0	0	0	0	2
	2	43	3	2	2	2	1	3	11
	3	89	8	4	0	11	5	3	20

of those with two polar bodies. However, it is not certain here that the two polar bodies represent the first and second polar bodies. To check this point more carefully the isolation was begun at the time of appearance of the first polar body, eggs with no polar bodies being separated from those with one. Then, at the time of appearance of the second polar body, eggs with two, one, or none were separated from the first group, and eggs with three, two or one from the second. The results of five experiments are summarized in Table III. Of 60 eggs that at first showed no polar bodies, nine later produced one polar body and twelve produced two. Of 134 eggs that at first had one polar body, 43 later showed two and 89 showed three. Actually some of the eggs isolated as having two polar bodies may later have formed three, since this was not carefully checked in each case. However, there are no significant

differences in the behavior of these latter two classes.[3] It may be seen from the table that at 1½ to 2½ hours after the start of the treatment in the first group cleavage is obtained in 38 out of the 39 eggs with no polar bodies, in all of the 9 eggs with one polar body and in all of the 12 eggs with two polar bodies. In the second group the two eggs with one polar body both divide at this time, but only 7 out of the 43 with two polar bodies and 12 out of the 89 with three polar bodies. Later, at 3½ to 5 hours, more cleavage is obtained in these last two classes, 17 out of the 43 with two polar bodies and 39 out of the 89 with three polar bodies. Thus, while practically all of the eggs with no polar bodies in the first group divide, only 14 per cent of the eggs with two and three polar bodies in the second group divide at the same time. Considerably later 42 per cent of the latter divide, but it is questionable whether, in most of these, anything like real cleavage had occurred (see below).

The type of cleavage that the eggs undergo is also given in Table III. It may be seen that most of the eggs that extrude no polar bodies divide at once into four cells. The four eggs, listed in the table, that divided into two cells and the ten that went into three cells were all eggs that had attempted to go into four cells. These eggs all show in the living condition, shortly after the time at which the second polar body should appear, four distinct nuclei (Figs. 13 and 15), which quite evidently result from submerged maturation divisions. The nuclei are visible for about 10 minutes, but it is apparent that the four centers for the first division arise from the centers associated with each of these nuclei. These centers evidently do not divide before the first cleavage, since the division is into four, not eight cells.

The eggs that extrude one polar body divide into three cells, the two eggs listed that went into two cells being derived from an attempted three-cell cleavage and the one egg that went into four being an exception. Since this type of egg is isolated from eggs that showed no polar body formation at the time when the first should appear, the single polar body is evidently the second polar body. In the living condition (Figs. 9 and 11) three nuclei are seen shortly after the time of extrusion of the second polar body.

The eggs of this group that extrude two polar bodies divide into two cells, one exception having gone into three. The two polar bodies of these eggs are both seen to arise simultaneously at the time of second polar body extrusion and evidently represent two "second polar" bodies. That is, the first maturation division was submerged, then each of the nuclei produced a polar body at the time of second polar body

[3] In normally fertilized eggs of *Urechis* the first polar body often fails to divide.

extrusion. The two polar bodies of such an egg (Fig. 5) are usually further apart than in the case of a first and second polar body (Fig. 2). Two nuclei later appear in these eggs. If their history were not known such eggs might be assumed to have normal first and second polar bodies, or to have simply a divided first polar body.

In the second group, two of the 134 eggs that had extruded the first polar body failed to extrude the second. These were isolated rather late and are doubtful cases. It appears from this as well as other observations that when the first polar body is extruded, the second almost immediately follows. Those eggs of this group that divided went for the most part into two cells at once. Many of them stop in the two, three or four-cell stage and in most of those listed in the table as having more than four cells, cleavage had occurred in only one of the original cells. Very often, too, it becomes difficult to decide in the living condition whether cleavage or some sort of fragmentation or lobulation had occurred. About two-thirds of the "two and three polar body" eggs that were listed as cleaved at the later time in Table III were actually in the condition illustrated in Fig. 12, showing small lobules. A more detailed study of these eggs would be necessary in order to decide whether cleavage had actually occurred. The number of lobes present is generally considerably greater than the number of cells to be expected at that time.

PLATE I

Explanation of Figures

Artificially activated eggs of Urechis; eggs of Figs. 1 and 2 treated for 5 minutes with 0.01n NH_3 in sea water; those of Figs. 3 to 6 and 9 to 16 treated for 15 minutes with the same solution; those of Figs. 7 and 8 treated for 5 minutes with 0.004n NH_3 in 40 per cent sea water.

FIG. 1. Showing first polar body extruded at the normal time.

FIG. 2. Same egg with second polar body.

FIG. 3. Blisters appearing on an egg at the time of polar body appearance.

FIG. 4. Same egg photographed in the same position ten minutes later, showing disappearance of blisters.

FIG. 5. Showing two polar bodies extruded simultaneously at the time when the second polar body should normally appear; the two nuclei present in this type of egg not visible here. The two polar bodies are further apart than in the case of a first and second polar body.

FIG. 6. First cleavage of the same egg shown in Fig. 5.

FIG. 7. "Poorly activated" type of egg.

FIG. 8. First cleavage of the same egg shown in Fig. 7.

FIG. 9. Showing one polar body extruded at the time when the second polar body should normally appear; the three nuclei remaining in the egg are visible.

FIG. 10. First cleavage of the same egg; polar view.

FIG. 11. Another egg showing three nuclei present after the time when the second polar body normally appears; a single polar body is present but not in focus.

FIG. 12. "Lobulation" of an egg in which polar body extrusion was apparently normal; taken when other eggs of the same lot were in 16 to 64 cells.

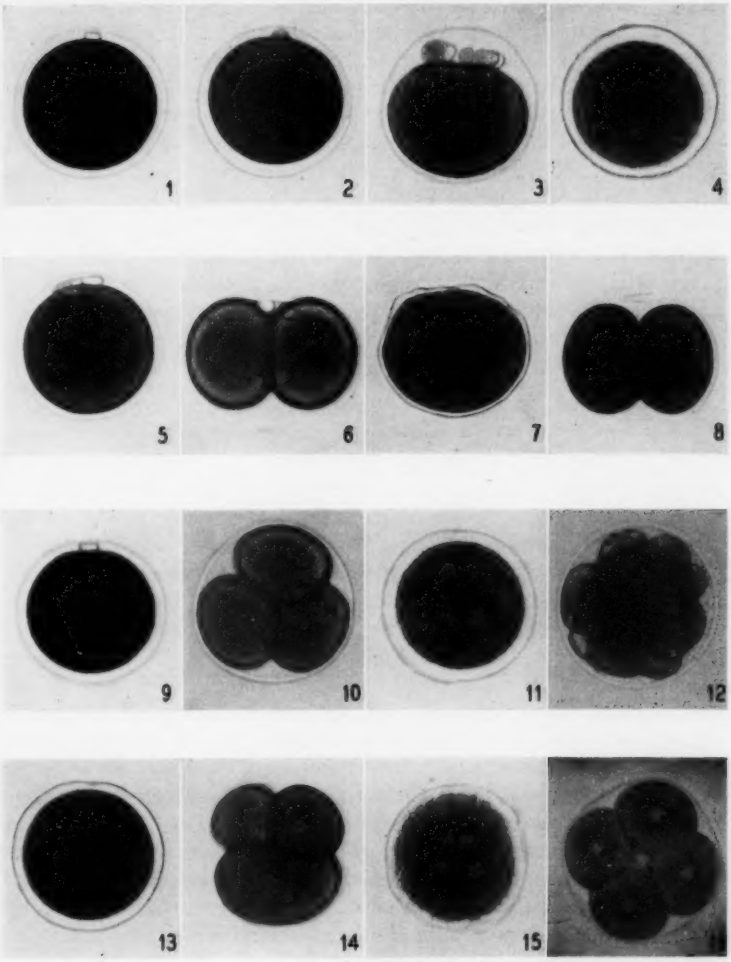

PLATE I

FIG. 13. Egg with four nuclei present after the time when the second polar body normally appears; no polar bodies are present but the pole is indicated by the slight dent in the membrane.

FIG. 14. First cleavage of the same egg, polar view; the three cells result from a disappearance of the cleavage plane between two cells.

FIG. 15. Another egg showing four nuclei present after the time when the second polar body normally appears.

FIG. 16. First cleavage of another egg of this type.

Time of Polar Body Extrusion

The time of appearance of the polar bodies varies with the length of exposure. This is illustrated in Table IV, in which the time of appearance of the first polar body is given. The time from the start of the treatment becomes increasingly greater, the longer the exposure to the activating solution. If the length of exposure is subtracted, we might expect the same value for all lengths of treatment. This, however, is not the case, as the last column in Table IV shows. The time first decreases with increasing length, then rises slightly. We may interpret this to mean that about four to five minutes of developmental progress is made during the sojourn in the solution. Thus, for exposures up to five minutes, the first polar body appears at approximately

Table IV

Time of extrusion of first polar body. Eggs treated with 0.01n NH_3 in sea water.

Length of Exposure	Time from Start of Treatment	Time from Removal from Solution
minutes	*minutes*	*minutes*
1	34½	33½
2	34	32
3	34	31
5	35	30
7	36	29
10	38½	28½
15	44	29
20	50	30

the same time from the start, and for longer exposures the delay in appearance of the first polar body corresponds to the additional time of treatment. This differs from the results with dilute sea water in which, if allowance is made for the treatment, the time of polar body extrusion is the same for all exposures (Tyler, 1931*a*). However, no developmental changes take place in the hypotonic sea water, whereas in the ammoniacal sea water breakdown of the germinal vesicle and membrane elevation is seen to occur. These changes occur in the solution at about the same rate as in normally fertilized eggs. It appears then that in the case of the longer exposures the observed changes occurring in the solution correspond to considerably more than the four or five minutes necessary to account for the delay in appearance of the first polar body. This means then that while certain changes such as membrane elevation proceed at a normal rate in the solution, other processes leading to polar body extrusion are blocked after four or five minutes. Without elaborating on this, it may be pointed out that a

possible explanation of the increasing percentage of eggs that fail to extrude polar bodies after increasing lengths of exposure may well be the lack of synchrony of the early changes.

It may also be suspected that in those cases in which cleavage is obtained after polar body extrusion the maturation divisions do not proceed normally, but that some of the chromosomes may fail to reach the maturation spindles or that the polar bodies may not receive a full complement. Evidence of this is contained in the work of Hiraiwa and Kawamura, who find often more than the haploid number of chromosomes on the first cleavage amphiaster and also polar bodies devoid of chromosomes. To establish this point, however, it would be necessary to show that no normal maturation divisions occur after treatments giving 100 per cent cleavage, or, where less cleavage is obtained, that a corresponding percentage of abnormal maturation divisions occurs. It may be possible to determine this in the cytological investigation.

Double Treatment

After certain lengths of exposure to ammoniacal sea water 100 per cent activation with normal polar body extrusion is obtained. No cleavage, however, occurs (Table I). Hiraiwa and Kawamura report for *Urechis unicinctus* that if such eggs are given a second treatment with ammoniacal sea water immediately after the extrusion of the second polar body, as much as 37 per cent cleavage may be obtained. We have tried to repeat this but with no success. In Table V three sets of re-treatment experiments are presented. In the first set the second series of exposures is given at various times ranging from 2 to 64 minutes after extrusion of the second polar body. With the exception of the first series of second exposures, less cleavage is obtained than by the single treatment. The slightly higher percentage of cleavage obtained in the series started at two minutes may be significant. The time of extrusion of the second polar body is taken when about 50 per cent have reached that stage. Eggs developing in an unshaken vessel show considerable spread in the time of polar body extrusion, which at 20° C. may be as much as four or five minutes and much more at lower temperatures. It is likely then that in the series begun at two minutes, a number of the eggs had not extruded the second polar body. In the second set in Table V the second treatment is begun at 11 minutes before the extrusion of the second polar body. In this case 5 per cent cleavage is obtained after 15 minutes of second treatment whereas the first treatment gave no cleavage. In the third set hypertonic sea water is used for the second treatment, but no increase in cleavage is obtained.

Three other sets of experiments similar to the first of Table V and using 0.003n, 0.005n and 0.01n NH_3 in sea water for the second treatment, gave similar results.

That cleavage may be obtained after a second treatment applied before the extrusion of the second polar body was shown in the hypotonic sea water experiments (Tyler, 1932*b*). But in those experiments, too, a second treatment applied after polar body extrusion gave

TABLE V

Double treatment. Solution A = 0.005n NH_3 in sea water. Solution B = 0.01n NH_3 in sea water. Solution C = 3.0 g. NaCl in 100 cc. sea water. Temperature 20.1 ± 1° C.

Treatment	Solution	Exposure	Activation	Time after second polar body extrusion of start of treatment	Cleavage
		minutes	*per cent*	*minutes*	*per cent*
First.....	A	5	99	—	2
Second.....	A	5 to 40*		2	2 to 4
Second.....	A	5 to 40*		6	1 to 2
Second.....	A	5 to 40*		12	1
Second.....	A	5 to 40*		20	1
Second.....	A	40		64	1
First.....	B	2	100	—	0
Second.....	B	1 to 5½		−11	0 to 0.1
Second.....	B	10		−11	2
Second.....	B	15		−11	5
First.....	A	5½	99	—	0.5
Second.....	C	2		3	0.5
Second.....	C	5 to 40*		3	0.5 to 0
Second.....	C	2		15	0.3
Second.....	C	5 to 30*		15	0.2 to 0

* Five-minute intervals.

no cleavage. Negative results, of course, do not prove very much. It is entirely possible that *U. caupo* and *U. unicinctus* respond differently to identical treatments, and that by varying such factors as the time treatment, the length of exposure, the temperature, the concentration and the kind of activating agent, cleavage might also occur in *U. caupo*. It is also possible that in the experiments of Hiraiwa and Kawamura some of the eggs had not given off the second polar body when the second treatment was begun.

Treatment with Ammoniacal-dilute Sea Water

Hypotonic sea water is not always effective in activating *Urechis* eggs. It generally fails to activate eggs that have stood in a dish for more than two hours, or that have been taken from animals kept in the laboratory more than a week. If, however, ammonia is added to the dilute sea water 100 per cent activation and a high percentage of cleavage may be obtained. An experiment with 0.004n NH_3 in 40 per cent sea water is given in Table VI. After five minutes exposure 100 per cent

Table VI

Ammoniacal hypotonic sea water. Solution = 0.004n NH_3 in 40 per cent sea water. Temperature = 20° C.

Exposure	Activation	Polar bodies			Cleavage		
		0	1	2 or 3	2-cells	3-cells	4-cells
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
3	90	98	1	1	85	0.1	0.1
4	95	95	2	3	95	1	0.5
5	100	97	1	2	90	0.2	0.1

activation is obtained and 90 per cent of the eggs divide. Very few (3 per cent) of the eggs extrude polar bodies and it is apparently only the "no polar body" eggs that divide. The first cleavage, however, is into two cells (Fig. 8). The eggs resemble the "poorly activated" eggs obtained by the short treatment with ammoniacal sea water or the straight dilute sea water treatment. These eggs, too, show rather poor membrane elevation until the time of first cleavage (Fig. 7). With other agents, this type of egg is obtained principally when the total activation is low. Here, however, with treatment giving 100 per cent activation, practically all of the eggs are of that type.

Activation of "Blocked" Eggs

It has been shown (Tyler and Schultz, 1932) that fertilization may be reversed in *Urechis* by treatment with acidified sea water within three minutes after insemination. The spermatozoa remain in such eggs, but they can be re-inseminated and thereupon they behave as polyspermic eggs. If, instead of re-inseminating, the eggs are given the kind of treatment with dilute sea water by which polar body extrusion but no cleavage is obtained, they develop as normally fertilized eggs. This experiment was repeated using ammoniacal sea water and the results are shown in Table VII. The control eggs (B) are placed

in sea water removed from the blocked eggs in order to check the possibility that the ammoniacal sea water merely stimulates the extra sperm present in the dish. The results show that this is not the case, since no cleavage is obtained after the four minutes exposure of the control eggs. From the blocked eggs, however, 80 per cent cleavage and 60 per cent normal development are obtained. With the longer treatment 10 per cent of the control eggs divide and 60 per cent of the blocked ones. The percentage of normal development is lower in this case, indicating some deleterious action of the longer treatment.

These results show that the treatment giving 100 per cent activation

TABLE VII

Activation of "Blocked" Eggs

Set A. Eggs placed in acid sea water (0.2 cc. of 0.5n HCl in 100 cc. sea water) at 1 minute after insemination. Removed after 20 minutes.

Set B. Unfertilized eggs placed in sea water taken from A. Both sets treated with 0.01n NH_3 in sea water.

Set	Exposure	Activation	Cleavage	Normal embryos
	minutes	*per cent*	*per cent*	*per cent*
A	4	100	80	60
B	4	100	0	0
A	10	100	60	20
B	10	100	10	0

and polar body extrusion but no cleavage actually provides sufficient stimulus for development. The ability of the blocked egg to divide is not due to any additional stimulus by the spermatozoön present but rather to the ability of the sperm aster to form an amphiaster.

Another kind of experiment shows the non-additive nature of the stimuli provided by the sperm and by artificial activation. If after treatment with dilute sea water or ammoniacal sea water sufficient to induce 100 per cent polar body extrusion but no cleavage, the eggs are transferred to a sperm suspension, fertilization, normal maturation, and normal development occurs. The combined action of the activating agent and the sperm is therefore not equivalent to a prolonged treatment with the activating agent, but simply equivalent to insemination of untreated eggs.

ACTIVATION WITH ACIDIFIED SEA WATER

Lefèvre (1907) activated eggs of *Thalassema* by means of acid and reported obtaining cleavage with or without polar body extrusion.

The solutions employed by Lefèvre were tried on *Urechis* eggs and were found to be strong enough to kill or injure the eggs in one or two minutes. Using weaker solutions good activation could be obtained. In Table VIII an experiment with 0.25 cc. of 1.0n HCl in 100 cc. sea water is presented. This is just about the lowest concentration of acid that gives activation, 0.2 cc. being too weak even after long exposures. It may be seen from the table that the activation rises rapidly with length of exposure and at the same time the percentage of cleavage drops off. It is almost exclusively the " poorly activated " type of egg that divides. These eggs, of course, produce no polar bodies. With

TABLE VIII

Activation with acidified sea water. Solution = 0.25 cc. of 1.0n HCl in 100 cc. sea water. Temperature = 20° C.

Exposure	Total activation	"Poorly" activated	Polar bodies			Cleavage		
			0	1	2 or 3	2-cells	3-cells	4-cells
minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0	—	—	—	—	—	—	—
2	75	95	95	2	3	60	10	0.2
3	100	25	15	15	70	12	0	0
5	100	10	5	10	85	5	0	0
7	100	0	1	4	95	0	0	0
10 to 30	100	0	0	0	100	0	0	0

other acids and other concentrations the results are similar. If three times the above concentration of acid is used, 100 per cent polar body extrusion with no cleavage is obtained after one-half-minute exposure.

DISCUSSION

It should be pointed out that we do not consider it impossible to obtain cleavage and normal development after extrusion of both polar bodies in *Urechis*. The point to be made, however, is that when eggs with no polar bodies and with two polar bodies are isolated from the same treated lot, practically all of the former divide whereas very few of the latter show real cleavage. In the " no polar body " eggs obtained by prolonged exposure to ammoniacal sea water, it is clear from the presence of four nuclei that submerged maturation divisions had occurred. In the " no polar body " eggs obtained by short exposure to ammoniacal sea water or to ammoniacal-dilute sea water the behavior is similar to that reported for dilute sea-water activation, no maturation

divisions occurring until the first cleavage. The first type divides into four cells at once, the second type into two.

The behavior of the chromosomes during cleavage in the first type of egg has not yet been worked out. In the second type both haploid and diploid cleavages have been found, although the embryos examined show only the diploid number (Tyler, 1932*a*). In a recent article on artificially activated frog's eggs Parmenter (1933) lists among the possible methods of regulation to diploidy this utilization of what is the equivalent of a maturation division spindle for the first cleavage. But it should be noted that if reduction proceeded normally in the first two cleavages only the haploid number of chromosomes would result. Since diploid as well as haploid later cleavages are observed, regulation must occur but this type of behavior certainly does not insure diploidy.

The same treatment that results in both maturation divisions occurring submerged also gives eggs in which only the first spindle is submerged. These eggs may produce either one or two polar bodies at the time of second polar body extrusion, and practically all the eggs cleave. When one polar body is produced three nuclei remain in the egg (visible for a short time) and the first cleavage is into three cells. If two polar bodies are produced two nuclei remain in the egg and the first cleavage is into two cells. Eggs that produce the first polar body almost invariably extrude the second. One should expect this to be the case from the manner of treatment. With the shorter exposures the polar bodies are extruded normally in all the eggs. With longer exposures changes are evidently produced in the egg that prevent polar body extrusion, even when the treatment ends before the time of first polar body formation. If the first polar body appears it simply means that these changes have not been produced or that the egg has recovered before that time. The second polar body should therefore follow. The extrusion of one or two second polar bodies after a submerged first division would mean a recovery of the egg in the interim. On this basis eggs in which the first division is submerged should occur after lengths of treatment that are intermediate between those giving only normal polar body formation and those giving only submergence of both maturation divisions. This is in fact the case.

The eggs that extrude two polar bodies have evidently received an adequate stimulus for development, as the experiments with "blocked" eggs show. That they generally fail to divide in our experiments is probably due to the inability of the centrosome left in the egg after polar body extrusion to divide. Those cases of cleavage after polar body extrusion may then result from division of this centrosome. In the four-cell stage of the "no polar body" eggs where the polar spindles

appear to serve for cleavage, the centrosome of one of the cells is equivalent to the one left in the egg after normal polar body extrusion. Yet in this case it continues to divide. The difference may be merely one of position in the cell. Centrifugation, in view of the experiments of Morgan and Tyler (1935), might be of use in examining these questions.

In the dilute sea-water experiments the type of " no polar body " egg with submerged maturation divisions was not obtained. The difference in the reaction of the egg while in the dilute sea water and in the ammoniacal sea water may account for this. With the former agent no developmental changes occur until removal to sea water. With the latter, development starts in the solution. Short exposures to the latter agent give results similar to those by dilute sea water. Prolonged exposures allow the development of the polar spindle to proceed, but either by modifying the surface or in some way preventing the movement of the spindle to the surface do not result in polar body extrusion. This effect is not produced by dilute sea-water activation since no developmental changes occur in the agent. The interest in activation by dilute sea water concerned primarily this fact. With all other parthenogenetic agents, continued exposure after the maximum of activation is reached soon results in injury and death. With dilute sea water in certain concentrations, continued exposure after the maximum of activation results in a falling-off of the activation to zero without injuring the eggs or affecting even their fertilizability.

Summary

1. *Urechis* eggs activated by prolonged exposure to ammoniacal sea water give a high percentage of cleavage as Hiraiwa and Kawamura showed, but such exposures also give a high percentage of polar body suppression.

2. Isolation of eggs of known polar body history shows that practically all those eggs divide that fail to extrude the first or the first and second polar bodies, but only a small percentage of those eggs that extrude the first and second polar body.

3. Eggs that fail to extrude the first polar body may produce two, one or no polar bodies at the time when the second polar body should appear. Later, two, three or four nuclei are visible in the egg corresponding to whether two, one or no polar bodies are present. The first cleavage of these three types gives two, three or four cells respectively.

4. Eggs that extrude the first polar body almost invariably produce the second.

5. Determinations of the time of polar body appearance after various lengths of exposure indicate that not more than four or five minutes progress toward polar body extrusion is made during sojourn in the solution, whereas other developmental changes progress further.

6. Eggs that had been given the kind of first treatment that results in 100 per cent normal polar body formation but no cleavage could not be induced to divide by a second treatment applied at various times after the extrusion of the second polar body. Cleavage was, however, obtained when the second treatment was started before the time of extrusion of the second polar body.

7. The "poorly activated" type of egg that divides without polar body formation or evidence of submerged maturation division is obtained by short exposures to ammoniacal sea water, to ammoniacal-dilute sea water, or to acid sea water, the second agent giving the highest percentages. The first cleavage is into two cells.

8. Normal development is obtained when "blocked" fertilized eggs are activated with the treatment that ordinarily results in 100 per cent polar body formation but no cleavage. Also superposition of fertilization after such treatment results in normal development.

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CROSS-REACTIVITY OF VARIOUS HEMOCYANINS WITH SPECIAL REFERENCE TO THE BLOOD PROTEINS OF THE BLACK WIDOW SPIDER

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It was reported by Graham-Smith in Nuttall's well known book (1904) that an antiserum against the blood of *Limulus* reacted with spider serum, even more strongly than with crab serum, but neither the species of spider nor any further details were given. Since Nuttall's sera were prepared by injecting rabbits with the whole blood serum of the species studied, it remained uncertain if the cross-reaction of *Limulus* and the spider were due to serologically similar blood pigments or to small amounts of some other protein.

I have found that an antiserum prepared by injecting the carefully purified hemocyanin of *Limulus* into rabbits (Hooker and Boyd, 1936) reacted with the diluted serum of the black widow spider. The only other species tested, outside of the injected antigen, which reacted with this antiserum was crab (*Cancer irroratus*), which gave a weaker reaction. The cross-reactions of a number of hemocyanins are set forth in Table I.

It will be noted that no other antiserum reacted definitely with the spider serum, and that the anti-crab serum did not react definitely with the *Limulus* hemocyanin, so that the relationship between these two antisera and antigens is not reciprocal, as is often the case. Of the other hemocyanins, *Cancer* and *Homarus* cross-react to a considerable degree, as do *Vivipara* and *Busycon,* paralleling their relationship.

Absorption of the anti-*Limulus* serum with the optimal (Dean and Webb, 1926) dose of spider serum removed all reactivity to spider but left considerable reactivity to *Limulus;* the limiting titer of the antiserum against *Limulus* remaining about the same. By determination of the optimal proportions ratio, which provides an index of the antibody content (the larger the ratio, the lower the content of antibody), it was possible to show that considerable antibody had been removed (the ratio rose from 780 to 2970). This phenomenon of partial cross-reactivity in related species is familiar (Hooker and Boyd, 1934), and could probably be made the basis of a quantitative measure of serological rela-

tionship. The complete removal of all reactivity to one antigen, leaving some to another, presumably indicates a common or closely related antigenic determinant in the two proteins, although in the present case it must be remembered that ultracentrifugal analysis of *Limulus* hemocyanin has revealed three distinct proteins with different molecular weights, and possibly only one or two of these might be concerned in the present cross-reaction. In the case of species as closely related as the hen and duck, absorption of the antiserum for one crystalline egg albumin with the other in most cases hardly affected the optimal ratio against the injected antigen (Hooker and Boyd, 1934); a greater effect would have been expected.

Determinations of copper and nitrogen (for methods, see Hooker and Boyd, 1936) on the diluted spider serum showed the presence of

TABLE I

Cross-reactivity by the precipitin test of various hemocyanins

Antiserum against purified hemocyanin of:	Antigen: Hemocyanins of:					
	Black widow spider	*Limulus*	*Homarus*	*Cancer*	Snail (*Vivipara*)	*Busycon*
Limulus	+	++	−	+	−	−
Homarus	−	−	++	+	−	−
Cancer	?	?	+	++	−	−
Vivipara	−	−	−	−	++	+
Busycon	−	−	−	−	+	++

The tests were made by the interfacial (ring) technic. The symbol − means no reaction, ? means doubtful reaction, + moderate reaction, ++ very strong reaction.

0.522 mg. of N per cc., and 0.0054 mg. Cu per cc. This gives a ratio nitrogen to copper of 96.7, not very different from the ratio of nitrogen to copper of 103 which follows from the values for *Limulus* hemocyanin (Redfield, 1930, Redfield et al., 1928). In some *Limulus* preparations I have found values as low as this or slightly lower. Montgomery (1930) says "In the case of *Limulus*, the hemocyanin appears to account for about 95 per cent of the protein of the serum." It would seem that the above data are consistent with the view that the serum protein of the black widow spider is chiefly or entirely a hemocyanin having about the same copper and nitrogen content as that of *Limulus*, which it closely resembles serologically. According to D'Amour et al. (1936), however, others have reported the presence of hemolysins in the legs of spiders, which presumably means that there may be present in the blood small amounts also of these substances, probably proteins.

Unfortunately it was impossible to estimate accurately the dilution of spider serum which resulted from bleeding the spiders used in this experiment. It was probably around 1 : 10 or 1 : 15. This would imply that the blood of the black widow spider contains 3 to 5 per cent of hemocyanin.

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